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STUDIES ON REACTIONS RELATING TO CARBOHYDRATES AND POLYSACCHARIDES.

XLV. THE POLYMERIZATION, UNDER THE INFLUENCE OF HEAT, OF CERTAIN COMPOUNDS CONTAINING THE ETHYLENE OXIDE RING[1]

BY EDWIN C. JAHN[2] AND HAROLD HIBBERT[3]

Abstract

The tendency of certain glycidol and glycoloside derivatives to polymerize under the influence of heat has been investigated. Phenyl glycidol, methyl glycidol, and 2 : 3, 6 : 7-di-epoxy-4, 5-dihydroxy octane all undergo polymerization. With phenyl glycidol acetate the tendency is much less pronounced, while polymerization does not occur with phenyl glycidol methyl ether. Apparently the phenomenon is associated with the presence of a hydrogen atom capable of migration. Polymerized phenyl glycidol was found to have an open-chain structure containing one ethylene oxide ring. 2-Methoxy ethyl glycoloside was obtained as a stable dimer, which appears to have a dioxane structure.

Introduction

In order to obtain a better understanding of the structure of cellulose and other polysaccharides, it seemed desirable to determine the manner in which simpler compounds containing *typical groups* unite to form larger molecules.

The hypothesis has been advanced that polysaccharides are derived from a simple building unit through the medium of residual valence forces, the seat of which lies in the oxygen ring (1, 2, 5, 19, pp. 576-604, 20). On the other hand rather convincing evidence has been presented that certain carbohydrate derivatives, for example 2, 3, 4-trimethyl arabonolactone, polymerize as a result of ring fission of an unstable oxygen ring, this leading to molecular combination by means of normal valency forces (12). Experimental evidence indicates that inulin is derived similarly from anhydrofructose units (17). This "normal valency" theory of polymerization has been extended to include all polysaccharides (14, 15, 16, pp. 74-96, 29, 30, 31).

It is not impossible that each of these factors may play a part in the formation of polymers from oxygen ring compounds. Variations in the structure

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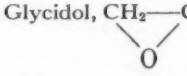
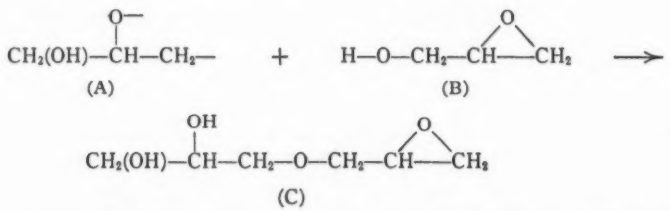
of the molecule, other than the nature and size of the ring, may also have a determining influence on the tendency towards polymerization, or association, and on the mechanism of the process.

Levene and Walti (24-28) studied various glycidol derivatives having certain constitutional relationships to the sugars, in an attempt to obtain data capable of being applied to an interpretation of the structure and mechanism of formation of the natural polysaccharides. No generalizations can be made as yet, however, other than that in the individual cases examined, differences in mode of combination occur, due probably to variations in molecular structure having a direct influence on ring stability.

Glycidol,  , when heated in a sealed tube, was found by

Nef (32) to undergo transformation into a resin. He assumed that this complex product was a polymerized glycidol chain compound formed as follows:



(C) then undergoes ring fission, and combines with another molecule of (B), the process thus giving rise to a chain polymer containing one terminal ethylene oxide ring.

The recent work of Levene and Walti (24, 25, 27) on propylene oxide, glycidol and glycidol acetate, has provided experimental evidence in support of this view.

In the earlier literature dealing with the simple carbohydrate type of compounds such as glycollic aldehyde, acetol, glyceric aldehyde, and dihydroxyacetone, Fenton (13), Bertrand (9, 11), Wohl (34, 35), Nef (32) and Kling (23) presented evidence indicating an equilibrium between the monomolecular and bimolecular forms of these compounds.

Investigations by Bergmann and coworkers (1–7) on the molecular weight of various lactolides, such as the methyl lactolide of acetol; ethyl glycoloside; glycolaldehyde-lactol acetate; methyl lactolide of cyclohexanol (2)-on-(1); acetaldol-lactol acetate, etc., led to the conclusion that while these products in organic solvents had a molecular weight corresponding to a dimer, in the gaseous state, judged in the light of their vapor density determinations, they appeared to be monomolecular. This passage from the monomeric to the dimeric stage, and its converse, were assumed to take place under the influence of "residual" rather than "normal" valency forces. However, in a later paper, Bergmann and Miekeley (8) were able to show that the earlier

views were incorrect due to the fact that their previous determinations of the vapor densities were inaccurate, and they arrive at the conclusion that all the above products possess a normal valency structure derived from two molecules of the monomolecular form.

Sole reliance upon molecular weight values for determination of structure has been severely criticized (16, pp. 74-96).

The simple ethylene oxide ring compounds offer an inviting field for study, because of the reactivity of the ring and its simple structure, and also in view of the conflicting data regarding ring behavior during transformation to the supermolecular state. It is necessary to collect further evidence regarding the nature of polymerization of this class of compounds before any generalizations can be made.

In the present paper the tendency towards polymerization, or association, under the influence of heat, of the following compounds was studied:

I. *Analogues of Glycidol*

(a) Phenyl glycidol, $C_6H_5—CH——CH—CH_2OH$ (O bridge)

(b) Methyl glycidol, $CH_3—CH——CH—CH_2OH$ (O bridge)

(c) 2 : 3, 6 : 7-Di-epoxy-4, 5-dihydroxy octane

$$CH_3—CH——CH—CH(OH)—CH(OH)—CH——CH—CH_3$$

(O bridges)

II. *Phenyl Glycidol Acetate and Methyl Ether*

(a) $C_6H_5—CH——CH—CH_2—O—CO—CH_3$ (O bridge)

(b) $C_6H_5—CH——CH—CH_2—O—CH_3$ (O bridge)

III. *2-Methoxy Ethyl Glycoloside*

$$CH_2——CH—O—CH_2—CH_2—O—CH_3$$

(O bridge)

Discussion of Results

Polymerization of Glycidol Analogues

Phenyl glycidol, methyl glycidol and 2 : 3, 6 : 7-di-epoxy-4, 5-dihydroxy octane contain a free hydroxyl group in the same position relative to the oxide ring. They should, therefore, exhibit a similar tendency to polymerize under the influence of heat, as shown by glycidol, and this was found to be the case.

2 : 3, 6 : 7-Di-epoxy-4, 5-dihydroxy octane polymerizes more readily than phenyl or methyl glycidol, as would be expected from the "double glycidol" nature of its molecule. This substance could not be distilled unchanged from its reaction mixture due to the ease with which it resinified.

(a) *Phenyl Glycidol*

Phenyl glycidol when heated at 140-155° C. for 29 hr. changed to a very viscous orange-colored liquid which was essentially pure dimeric phenyl glycidol. When heated for a much longer period (32 days) in sealed tubes at 105° C. solid resins were obtained from which a colorless amorphous polymer could be isolated.

Methylation of the dimeric phenyl glycidol yielded a dimethoxy derivative. Addition of ammonia to this dimethoxy compound gave a product formed from one mole of ammonia and one mole of dimethoxy dimeric phenyl glycidol, thus proving the presence of only one ethylene oxide ring. Trityl chloride and the dimeric phenyl glycidol gave a monotrityl derivative (18), indicating the presence of only one primary hydroxyl group, and not two as would be the case, presumably, if the compound were an "associated product".

The addition of ammonia to the solid polymerized phenyl glycidol yielded amines which contained less than one atom of nitrogen per molecule of polymer. This indicated the presence of not more than one ethylene oxide ring, as was also found to be the case by Levene and Walti (24) with polyglycidol.

Polymerization of phenyl glycidol apparently takes place through a primary ring fission of one molecule and addition to this of a second unchanged molecule. The resulting polymer still contains one ethylene oxide ring, which in turn undergoes ring fission and then adds on a further molecule of the original glycidol.

(b) *Methyl Glycidol*

Methyl glycidol is converted into an open-chain dimer when heated at 105° C. in a sealed tube for 22 days. The latter reacts with phenyl isocyanate to form a di-phenyl urethane derivative which still contains an ethylene oxide group, indicating that methyl glycidol most probably polymerizes in the same manner as phenyl glycidol.

(c) *2 : 3, 6 : 7-Di-epoxy-4, 5-dihydroxy Octane*

Attempts to prepare and isolate 2 : 3, 6 : 7-di-epoxy-4, 5-dihydroxy octane by oxidation of dipropenyl glycol with benzoylhydroperoxide yielded almost entirely a resin. The monomeric form was most probably present in the reaction mixture because the theoretical amount of oxygen was consumed for the reaction, but under the influence of heat, it apparently undergoes polymerization very readily. The structure of this polymer has not yet been investigated. The molecular weight of the resin indicated that it was essentially a trimer.

Polymerization of Phenyl Glycidol Acetate and of Phenyl Glycidol Methyl Ether

The tendency for phenyl glycidol, phenyl glycidol acetate, and phenyl glycidol methyl ether to polymerize was found to decrease in the order named. This would seem to indicate an intimate relation between tendency to poly-

merize and the presence, or absence, of a hydrogen atom capable of migrating, since when this is replaced by a methyl group, as in the methyl ether, the tendency to undergo polymerization ceases.

Phenyl glycidol acetate was readily transformed into a very viscous yellow substance by heating for six days at 145-165° C. at atmospheric pressure, or for four weeks in a sealed tube at 140° C. The greater part of the reaction product was a solid red resin, apparently a polymerized phenyl glycidol acetate, having an average molecular weight of 623, and thus corresponding, approximately, to a trimer.

Phenyl glycidol methyl ether, when heated for a long period in a sealed tube with or without a catalyst, yielded only resins, oily liquids and water, indicating that decomposition with loss of water and condensation had occurred. None of the resins formed appeared to be polymers of phenyl glycidol methyl ether.

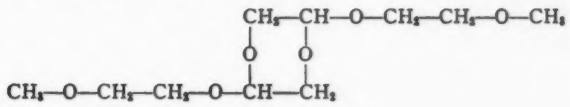
Polymerization of 2-Methoxy Ethyl Glycoloside

When methoxy ethyl vinyl ether ($CH_2=CH—O—CH_2—CH_2—O—CH_3$) was oxidized with benzoylhydroperoxide, only the dimeric 2-methoxy ethyl glycoloside (b.p., 118-119° C./0.1 mm.) could be isolated.

Apparently under the conditions of synthesis and distillation, the monomer undergoes dimerization. The dimer formed is quite stable towards heat for it may be redistilled nearly quantitatively (0.1 mm. pressure) without decomposition.

In alcoholic ammonia solution the dimer absorbed only a very small amount of ammonia. This may be due either to the presence of a small amount of an ethylene oxide form present, perhaps in equilibrium with a more stable form, or to the fact that the dimer, in the stable dioxane form, is slightly decomposed allowing addition of a small amount of ammonia.

The fact that no dissociation occurs when the dimer is distilled and that the product combines with only one-sixth of a mole of ammonia, instead of two moles, points to its structure as that of a stable dioxane derivative,



rather than an "associated" type of polymer.

Experimental Part

The oxidation of the unsaturated compounds to ethylene oxide derivatives was carried out in all cases by means of benzoylhydroperoxide (Prileschajew's reagent) (33). The procedure outlined by Hibbert and Burt (21) was followed with slight modifications.

Phenyl Glycidol and its Polymers

Preparation of Phenyl Glycidol

To a chloroform solution of benzoylhydroperoxide containing 6.2 gm. of available oxygen was added 49 gm. of cinnamyl alcohol, the temperature of the solution being kept at 0° C. Reaction was complete after 20 hr. Yield of liquid phenyl glycidol, 42.9 gm. (b.p., 134-135° C./4-5 mm.). n_D^{27}, 1.5427. It readily decolorized potassium permanganate solution but not bromine in carbon tetrachloride.

After standing 16 hr. at room temperature, the product solidified to a mass of wax-like crystals; m.p., 26.5° C. Analysis:—Found*: C, 71.86; H, 6.58%. Calcd. for $C_9H_{10}O_2$: C, 72.00; H, 6.66%. Mol. wt. ($C_2H_4Br_2$), 154.6; calcd., 150.

Phenyl Urethane Derivative

This was obtained by mixing together phenyl glycidol and phenyl isocyanate. After recrystallization from benzene-ligroin solution the product melted at 87° C. Found: C, 71.66, 71.77; H, 5.20, 5.47%. Calcd. for $C_{16}H_{15}O_3N$: C, 71.64; H, 5.57%.

Dimeric Phenyl Glycidol

Phenyl glycidol (6.5 gm.) was heated for 29 hr. at 140-155° C. The thick viscous mass so obtained was then heated at 100° C./15 mm., yielding in this way less than 0.1 gm. of distillate.

Analysis of the residual liquid showed it to be practically pure dimeric phenyl glycidol. n_D^{20}, 1.5744. Found: C, 72.08, 72.06; H, 6.77, 6.80%. Calcd. for ($C_{18}H_{20}O_4$): C, 72.00; H, 6.66%. Mol. wt. ($C_2H_4Br_2$), 328, 309; calcd., 300.

Trityl Derivative

One gram of the dimer was dissolved in 5 cc. of pyridine and 1.86 gm. of trityl chloride added. After 24 hr. the mixture was shaken thoroughly with water, the solid reaction product dissolved in benzene, and the product precipitated by adding ligroin. After three precipitations the trityl derivative was obtained as a micro-crystalline colorless product. Its alcoholic solution was readily decolorized by potassium permanganate. When dissolved in dry toluene and treated with clean sodium an evolution of gas occurred. Mol. wt. ($C_2H_4Br_2$); found, 536; calcd. for the monotrityl derivative of dimeric phenyl glycidol ($C_{37}H_{34}O_4$), 542.

Dimethyl Ether of Dimeric Phenyl Glycidol

The dimer (1.75 gm.), dissolved in 10 gm. of methyl iodide, was mixed with 7 gm. of silver oxide and 3 gm. of anhydrous sodium sulphate, and the mixture kept at 45-50° C. under reflux for 24 hr. The reaction product was filtered and the residue washed with ether. Removal of the ether and methyl iodide, followed by distillation of the residue, yielded 0.6 gm. of a colorless

* *The micro-method of analysis was used throughout this investigation.*

oil; b.p., 180-185° C./0.03 mm. n_D^{28}, 1.5383. The product readily decolorized permanganate solution. Found: CH_3O, 18.72%. Calcd. for $C_{20}H_{24}O_4$; 18.90%. Mol. wt. ($C_2H_4Br_2$), 314; calcd. 328.

The residue was a reddish viscous oil which could not be distilled. It contained 14.77% CH_3O and hence was only partially methylated.

Addition of ammonia. A portion (0.25 gm.) of the dimethyl ether of dimeric phenyl glycidol was dissolved in 50 cc. of alcoholic ammonia (saturated) and left standing in a sealed bottle for three days at 35-40° C. Removal of the alcohol and excess ammonia by vacuum distillation left a viscous orange-colored material. Found: N, 4.16%; calcd. for ($C_{20}H_{24}O_4+NH_3$), 4.06%.

Phenyl Glycidol Polymer

Phenyl glycidol (10 gm.) when heated in a sealed tube at 105° C. for 32 days yielded a solid orange-colored resin. This was purified by dissolving in 50 cc. of benzene and precipitating by the addition of 150 cc. of ligroin, the operation being repeated three times. The white fluffy powder so obtained decolorized cold potassium permanganate solution and in dry toluene reacted with sodium, evolving a gas. Found: C, 71.83; H, 6.90%. Calcd. for $(C_9H_{10}O_2)_x$: C, 72.00; H, 6.66%. Mol. wt. ($C_2H_4Br_2$), immediately after solution, 816; after 30 min., 496; after 24 hr., 438.

Addition of ammonia. The polymerized phenyl glycidol (0.7 gm.) when mixed with 150 cc. of alcoholic ammonia solution and allowed to stand for three days at 30° C., gave a solid yellow resin. Found: N, 0.343, 0.379%; calcd. for one amino nitrogen atom per molecular weight of 816; N, 1.68%. Thus considerably less than one molecule of ammonia is taken up per molecule of polymer, which, in the light of the molecular weight determinations, indicates that the actual molecular weight may be greater than 816, and that possibly dissociation occurs in solution.

Methyl Glycidol and its Dimer

Preparation of Methyl Glycidol

Crotyl alcohol (22 gm.; b.p., 116-119° C.) was treated with a chloroform solution of benzoylhydroperoxide containing 4 gm. of active oxygen for 24 hr. at 0° C. Yield of methyl glycidol, 3 gm. (b.p., 93-97° C./44 mm.).

Methyl glycidol is a mobile liquid with a pleasant odor. The phenyl urethane, crystallized from benzene-ligroin mixture, melted at 66.5° C. Found: C, 63.54, 63.99; H, 6.06, 5.96%. Calcd. for ($C_{11}H_{13}O_3N$): C, 63.77; H, 6.28%.

Polymerization of Methyl Glycidol by Heat

Methyl glycidol (0.5 gm.) on heating in a sealed tube at 105° C. for 22 days was converted into a viscous yellow liquid.

A small amount of this product was added to an equal weight of phenyl isocyanate and allowed to stand for three days when the mixture had changed to a clear yellow solid. By dissolving in benzene, precipitating with ligroin

and then again re-precipitating from ether solution, the diphenyl urethane derivative was obtained as a cream-colored powder; m.p., 78-80° C. This material decolorized dilute potassium permanganate solution, and, when dissolved in toluene, showed no action towards sodium. Analysis indicated that the substance was a nearly pure diphenyl urethane of dimeric methyl glycidol. Found: N, 6.22, 6.30%. Calcd. for monophenyl urethane derivative of dimeric methyl glycidol: N, 4.74%; for diphenyl derivative; N, 6.98%.

Oxidation of Dipropenyl Glycol with Benzoylhydroperoxide

Dipropenyl glycol (18 gm.; b.p., 105-109° C./5-6 mm.) was added to a chloroform solution of benzoylhydroperoxide containing 4.0 gm. of active oxygen. After standing two days at 0° C. the theoretical amount of oxygen had been absorbed.

Fractionation of the reaction mixture under reduced pressure yielded several small liquid fractions, one of which, boiling at 125-128° C./4 mm. (n_D^{23}, 1.4648), had a molecular weight of 190. Calcd. for 2 : 3, 6 : 7-di-epoxy-4, 5-dihydroxy octane, 174. On raising the temperature to 148° C./4 mm. most of the product was left in the distilling flask as a solid resin (10 gm.). This was purified by dissolving in 40 cc. of benzene and then precipitating by the addition of 75 cc. of ligroin, the operation being repeated three times. The final product possessed a yellowish brown color, and presumably still contained some impurity as is indicated also by the analysis. Found: C, 55.43, 55.67; H, 7.68, 7.62%. Calcd. for $(C_8H_{14}O_4)_x$: C, 55.17; H, 8.05%. Mol. wt. ($C_2H_4Br_2$), 431; calcd. for $(C_8H_{14}O_4)_3$, 422.

Phenyl Glycidol Acetate

Preparation and Properties

Cinnamyl acetate was prepared by acetylating 50 gm. of cinnamyl alcohol with 76 gm. of acetic anhydride and 10 gm. of sodium acetate. Colorless oily liquid; b.p., 135-138° C./9 mm. n_D^{22}, 1.5390. Yield, 86%.

The acetate (53 gm.) was added to a chloroform solution of benzoylhydroperoxide containing 6.0 gm. of active oxygen, and the mixture allowed to stand for three days at 0° C. The reaction product on fractionation yielded phenyl glycidol acetate as a heavy oil; b.p., 129-132° C./3 mm. n_D^{22}, 1.5208. Yield, 70%. It decolorized cold dilute potassium permanganate solution immediately. Found: C, 68.59; H, 6.58%. Calcd. for $C_{11}H_{12}O_3$: C, 68.75; H, 6.35%. Mol. wt. ($C_2H_4Br_2$), 190: (benzene), 183; calcd., 192.

Behavior on Heating

Experiment I. Phenyl glycidol acetate (5 gm.) when heated for six days at 145-165° C. at atmospheric pressure was converted into a very viscous substance.

Experiment II. Phenyl glycidol acetate (10 gm.) was heated in a sealed tube for four weeks at 140° C. The resulting product was a thick viscous liquid.

The products from Experiments I and II were mixed together, then dissolved in benzene, and ligroin added. The solid (A) separating out was filtered off, the solvent removed from the mother liquor and the residual oil (B) fractionated.

Fractionation of (B)

Fraction (1) 100-105° C./0.13 mm. Wt., 0.5 gm. n_D^{25}, 1.5141.
(2) 90-94° C./0.12 mm. Wt., 0.5 gm. $n_D^{24.5}$, 1.5199.
(3) 126-127° C./0.15 mm. Wt., 2.0 gm. $n_D^{24.5}$, 1.5144.
(4) 140-145° C./0.10 mm. Wt., 2.5 gm. $n_D^{24.5}$, 1.5377.

On analysis only fraction (4) was found to correspond empirically with phenyl glycidol acetate. Found: Fraction (4): C, 68.50, 68.43; H, 6.57, 6.66%. Calcd. for $(C_{11}H_{12}O_3)_x$: C, 68.75; H, 6.35%. Mol. wt. $(C_2H_4Br_2)$; 256: (benzene) 285; calcd. for $(C_{11}H_{12}O_3)_2$, 384.

The product (A) precipitated from the benzene-ligroin mixture (1 : 1) was a solid red resin (9 gm.). Found: C, 68.48; H, 6.51%. Calcd. for $(C_{11}H_{12}O_3)_x$: C, 68.75; H, 6.35%. Mol. wt. $(C_2H_4Br_2)$, 623. This material is apparently a polymer with an average molecular weight corresponding to three to four moles of phenyl glycidol acetate.

Phenyl Glycidol Methyl Ether

Preparation and Properties

Cinnamyl alcohol (40 gm.) was methylated with 130 gm. of methyl iodide and 180 gm. of silver oxide. The methyl ether was obtained as a liquid having a very pleasant odor. Yield, 87%; b.p., 92° C./5 mm. n_D^{20}, 1.5443. Mol. wt. $(C_2H_4Br_2)$, 147; calcd., 148.

Treatment of 36.1 gm. of methyl cinnamyl ether with benzoylhydroperoxide yielded phenyl glycidol methyl ether; b.p., 130-137° C./21-23 mm. n_D^{20}, 1.5170. Found: CH_3O, 18.83%. Calcd. for $(C_{10}H_{12}O_2)$, 18.90%. Mol. wt. $(C_2H_4Br_2)$, 163, 166; calcd., 164. Yield, 34 gm.

Behavior on Heating

Portions of phenyl glycidol methyl ether were heated in sealed tubes as follows: (*a*) 9.6 gm. with a trace of moist potassium hydroxide; (*b*) 4 gm. with a trace of moist zinc chloride; and (*c*) 4 gm. without a catalyst. The tubes were heated at 140-150° C. for 11 weeks, a thick reddish liquid being produced in each case. In (*b*) a small amount of water separated out.

The products from experiments (*a*) and (*c*) were mixed and fractionated:

Fraction (1) 94-96° C./3 mm. (wt. 4 gm.) n_D^{25}, 1.5100, colorless oily liquid.
(2) 100-105° C./0.05-0.06 mm. (wt. 1.0 gm.) $n_D^{24.5}$, 1.5617, heavy yellow liquid.
(3) 125-130° C./0.03 mm. (wt. 1.5 gm.) $n_D^{24.5}$, 1.5858, viscous yellow liquid.

The residual product was dissolved in benzene and a portion precipitated by the addition of ligroin. This treatment was repeated from benzene and

ether solutions, respectively, yielding 1.0 gm. of a light brown powder (Fraction 4). Evaporation of the solvent from the mother liquor yielded 3.0 gm. of a viscous red liquid (Fraction 5).

Found: Fraction (1) C, 69.96, 69.88; H, 7.65, 7.76%; CH_3O, 20.52, 20.31%. Calcd. for $(C_{10}H_{12}O_2)_x$: C, 73.17; H, 7.32; CH_3O, 18.90%. Fraction (2) CH_3O, 14.48%; Fraction (3) CH_3O, 12.61%; Fraction (4) CH_3O, 6.73%; Fraction (5) CH_3O, 10.91%.

Under the conditions indicated, phenyl glycidol methyl ether does not polymerize, but apparently undergoes other complex changes with formation of viscous and solid resins.

Dimeric 2-Methoxy Ethyl Glycoloside

Dimeric 2-methoxy ethyl glycoloside was prepared by the methylation of the sodium salt of hydroxy ethyl vinyl ether (22). Bromoethylidene glycol (75 gm.) and 150 cc. of pure dry ether were placed in a 500-cc. three-necked flask fitted with a mercury-seal stirrer and a reflux condenser with calcium chloride tube, and 21.5 gm. of sodium, cut in small pieces, introduced in one addition. The mixture was vigorously stirred and, after the reaction had subsided, the contents was heated at a temperature of 70-80° C. for seven hours. The bulk of the ether was then removed by distillation. Methyl iodide (77 gm.) was added and the mixture gently heated on a water bath with stirring for 16 hr. Water was then slowly added until the salts had dissolved. The aqueous solution was extracted twice with ether, the combined ether solutions washed with small amounts of water and dried over anhydrous potassium carbonate.

On fractionation 26.1 gm. of methoxy ethyl vinyl ether was obtained as a mobile, colorless liquid having a rather penetrating musty odor; b.p., 107-110° C./760 mm. n_D^{20}, 1.4130. Mol. wt. $(C_2H_4Br_2)$, 105; calcd. for $C_5H_{10}O_2$, 102.

Oxidation of Methoxy Ethyl Vinyl Ether

The ether (23.6 gm.) was oxidized with benzoylhydroperoxide containing 3.8 gm. of active oxygen. Fractionation of the reaction product yielded several small, indefinite fractions, and a large pale yellow liquid fraction (3.5 gm.). The latter, on redistillation, gave a colorless oil; b.p., 118-119° C./0.1 mm. n_D^{22}, 1.4674. Found: CH_3O, 26.40, 26.34%. Calcd. for $(C_5H_{10}O_2)_2$, 26.27%. Mol. wt. $(C_2H_4Br_2)$, 242; calcd, 236. The product was therefore a dimer of 2-methoxy ethyl glycoloside.

Addition of ammonia. Treatment with alcoholic ammonia gave a reddish oil. Found: N, 0.82, 0.95%. Calcd. for $(C_5H_{10}O_2)_2 + 2NH_3$, N, 10.37%; for $(C_5H_{10}O_2)_2 + NH_3$, N, 5.53%.

This result indicates that the dimer is not an associated compound containing two ethylene oxide rings, but more probably a stable dioxane

structure formed by the union of the two oxide rings. The small amount of ammonia reacting may indicate either an equilibrium with an ethylene oxide form, or be due to slight ring fission of the dioxane structure.

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THE ALKALOIDS OF FUMARACEOUS PLANTS

IV. *Adlumia fungosa*, GREENE

By Richard H. F. Manske

Abstract

An investigation of the alkaloids of *Adlumia fungosa* has confirmed the presence of *protopine*. The alkaloid previously reported as melting at 176-177° C. proved to be bicuculline. Two new alkaloids, adlumine, $C_{21}H_{21}O_6N$, and adlumidine, $C_{19}H_{17}O_6N$ (?) have been isolated. These names have been used because it is felt that the alkaloids described by Schlotterbeck under the same names were either impure or improperly analyzed. The investigation is being continued.

Schlotterbeck and Watkins (5) and Schlotterbeck (4) have recorded the isolation of five alkaloids from *Adlumia fungosa*, Greene (*A. cirrhosa* Raf.), two of which were identified as protopine and α-allocryptopine respectively. The other three bases were regarded as new. One, *adlumine*, was stated to possess the empirical formula, $C_{39}H_{41}O_{12}N$, and its melting point was given as 188° C. (? corr.). Another was named *adlumidine* and was regarded as being represented by $C_{30}H_{29}O_9N$; m.p., 234° C. (? corr.). The fifth was not named, and on account of the small quantity isolated, its composition was not determined, but it was stated to melt at 176-177° C. (? corr.).

Adlumia fungosa (N. O. Fumariaceae) is a handsome climbing biennial which under favorable conditions attains a length of 25 to 30 ft. It is widely distributed throughout eastern North America, but in general it is found in any one locality in only small amounts. It was therefore a matter of good fortune to find it in sufficient quantity for a chemical investigation, and in continuation of a program of research outlined previously (1) its collection and chemical examination were undertaken.

The presence of protopine was readily confirmed, but if α-allocryptopine is present at all in the available specimen, the amount is exceedingly minute, since the total uncrystallized mixture of bases from the mother liquors amounted to only a very small fraction of the total bases. The alkaloid melting at 176-177° C. was readily obtained and proved to be identical with bicuculline (2, 3).

In regard to Schlotterbeck's adlumine and adlumidine the situation is complicated by the unusual compositions ascribed by him to these bases. It is certain that no alkaloids of such composition were present in tractable amounts in the material under investigation. At the same time, however, an alkaloid melting at 235° C.* has now been obtained. Analyses yield figures which are in substantial agreement with $C_{19}H_{17}O_6N$ or with $C_{19}H_{15}O_6N$. Since the substance appears to be the same as that to which Schlotterbeck assigned the name adlumidine this name will be retained for it.

[1] *Manuscript received March 1, 1933.*
Contribution from the National Research Laboratories, Ottawa, Canada.

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**All melting points are corrected.*

Further, it is proposed to transfer (or retain) the name adlumine to an alkaloid melting at 180° C. which was obtained in the course of the present investigation, the empirical formula of which is $C_{21}H_{21}O_6N$. It is probable that Schlotterbeck's adlumine was the higher melting form of bicuculline (196° C.) contaminated with some adlumine. Such mixtures have been encountered in the present work and their separation by fractional crystallization was accomplished only in part.

During the various investigations of Fumaraceous plants which the author has completed in whole or in part, a unified procedure has been developed which appears to have certain advantages over the various methods hitherto described. Investigations in progress, dealing with *Dicentra eximia*, *Corydalis sempervirens* and *C. aurea* have shown the usefulness of the method, and since any modifications necessitated by special conditions are of a minor nature it seems desirable to describe the method in full in this communication. In subsequent publications it is proposed to refer merely to the specific labels which will be given to each fraction. It is thus hoped to avoid the lengthy repetition which would otherwise be necessary in each case.

Experimental

Preparation of the Extracts

The ground plant material is thoroughly extracted with methanol in a Soxhlet apparatus and the solvent largely distilled (Extract-E). Much hot water is added and the mixture rendered acidic to Congo red with hydrochloric acid. The remainder of the methanol is boiled off, preferably under reduced pressure and the mixture cooled. In general the resinous and fatty products are difficult to remove by filtration, but if the mixture is allowed to remain in an ice chest for several days or weeks the aqueous solution frequently becomes perfectly clear and can be separated from the residue (R) by decantation. The latter is again treated with very dilute boiling hydrochloric acid, and cooled as before and the two decantates combined (Aqueous Extracts-S).

In more difficult cases it may be necessary to add chloroform to the aqueous mixture and filter through a layer of charcoal, but this is not recommended if it can be avoided.

The aqueous solution (S) is treated with a little charcoal in the cold, filtered and thoroughly exhausted with chloroform. The combined extract (C) is evaporated to a convenient volume and clarified by filtration with a little charcoal. In general it is inadvisable to attempt to obtain pure products directly from this extract. The aqueous solution from which chloroform no longer removes appreciable amounts of extracts is filtered from a small amount of amorphous deposit (D) insoluble in both media (Filtrate-SC).

Examination of the Chloroform Extract (C)

The solvent is largely removed and the residue boiled with dilute hydrochloric acid. Separation of the insoluble residue (RC) may be effected either by filtration, decantation or ether extraction, depending largely upon its

properties. In any case the aqueous solution (SR) is exhausted with ether and yields the extract (LC) which, depending upon its nature, may or may not be combined with (RC). The ether is expelled from the aqueous solution and the latter basified with excess potassium hydroxide. When the precipitate (BC) has become granular it is filtered off and washed with water and the filtrate (FC) exhausted with ether (Extract-EC). The residual aqueous solution (CES) is saturated with carbon dioxide and the precipitated base (BCE) filtered off. The filtrate is again exhausted with ether and yields the extract (EEC).

Examination of the Filtrate (SC)

An excess of ammonia is added to this solution and the mixture thoroughly extracted with chloroform. When large quantities of extract are being manipulated it is frequently desirable to allow the basified solution to remain in contact with chloroform in a cold place for several days to allow the complete separation of ammonium magnesium phosphate and other inorganic substances. The mixture is then filtered and the insoluble residue thoroughly washed with chloroform. Separation of the chloroform layer is thus greatly facilitated in the subsequent extractions.

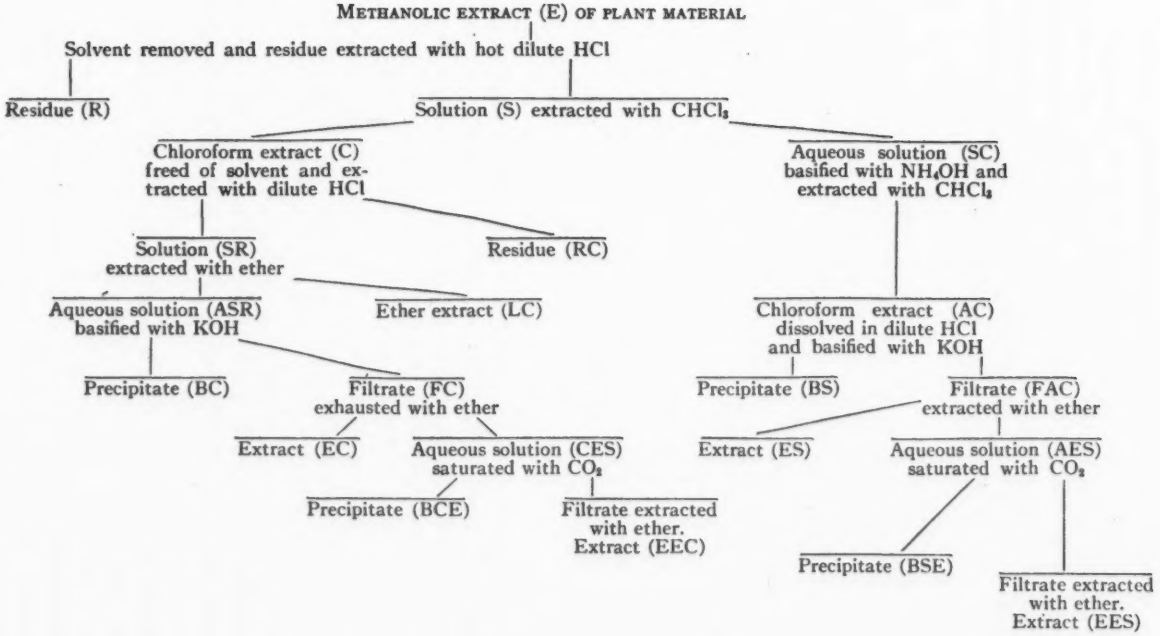
The combined chloroform extract (AC) is evaporated to a convenient volume and filtered through a layer of charcoal to remove some suspended matter. In general it is difficult to obtain crystalline products directly from this solution and for the purpose of further separation and purification the solvent is removed and the residue dissolved in hot dilute hydrochloric acid. A small amount of insoluble resin, which is generally present here, is removed with the aid of charcoal. The cooled filtrate is basified with excess potassium hydroxide and the precipitate (BS) filtered off after it has become granular or coherent and the filtrate (FAC) exhausted with ether (Extract-ES). The residual aqueous solution (AES) is saturated with carbon dioxide and the precipitated base (BSE) filtered off. The filtrate is again extracted with ether and yields the extract (EES).

It is to be pointed out that the separations above outlined while not strictly quantitative, nevertheless effect a fractionation which greatly facilitates the subsequent isolation and crystallization of pure individuals. The further treatment of each fraction is obviously dependent upon its properties and may be different for different plants.

The included flow sheet is included in the interests of clarity.

The author's experience does not enable him to allocate all or most of the known alkaloids of Fumaraceous and Papaveraceous plants in this scheme, but it may be of interest to review briefly where a number of them are found.

Dicentrine (and probably also glaucine) is found in the non-phenolic fraction (BC) of the chloroform extract (C). Corydine and isocorydine are removed from the alkaline solution by means of ether (EC). Bicuculline is precipitated only by means of carbon dioxide (BCE) and (EEC).


METHANOLIC EXTRACT (E) OF PLANT MATERIAL
Solvent removed and residue extracted with hot dilute HCl
Residue (R)
Solution (S) extracted with $CHCl_3$
Chloroform extract (C) freed of solvent and extracted with dilute HCl
Aqueous solution (SC) basified with NH_4OH and extracted with $CHCl_3$
Solution (SR) extracted with ether
Residue (RC)
Aqueous solution (ASR) basified with KOH
Ether extract (LC)
Chloroform extract (AC) dissolved in dilute HCl and basified with KOH
Precipitate (BC)
Filtrate (FC) exhausted with ether
Precipitate (BS)
Filtrate (FAC) extracted with ether
Extract (EC)
Aqueous solution (CES) saturated with CO_2
Extract (ES)
Aqueous solution (AES) saturated with CO_2
Precipitate (BCE)
Filtrate extracted with ether. Extract (EEC)
Precipitate (BSE)
Filtrate extracted with ether. Extract (EES)

The non-phenolic alkaloids (BS) contain the protopine and cryptopine, while bulbocapnine is not removable from the alkaline solution by means of ether. It is therefore found in the fraction (BSE).

Adlumia fungosa

There was available 11 kilos of dried plant of which 255 gm. was the underground portion. Each portion was examined separately, but since all the products to be described subsequently were found in both parts only the examination of the stems and leaves need be recorded.

Isolation of Fumaric Acid

The insoluble residue (R) was extracted with several successive portions of hot water and the combined extracts clarified with charcoal. On cooling, a copious deposit of fumaric acid was obtained. By means of several recrystallizations which included concentration of the mother liquors there was obtained 70 gm. of pure fumaric acid melting at 295° C. (286° C. uncorr.). Comparison with an authentic specimen of fumaric acid failed to disclose any differences.

Isolation of Bicuculline and Adlumine

The experience gained while working with the alkaloids from the roots showed that the chloroform extract (C) contained only these two alkaloids in tractable amounts and the same proved to be true in the case of the aerial portion.

The acid solution of the bases (ASR) was basified with ammonia and extracted with chloroform. The combined extracts were evaporated to a syrup and the latter repeatedly extracted with much ether until only an insignificant amorphous residue remained. Evaporation of the extract to a small volume yielded a thin syrup which gradually crystallized in contact with methanol. Examination with a lens disclosed the presence of two products which were separated by a long series of fractional crystallizations from a variety of solvents,—methanol, ethyl acetate, acetone, etc. From the more soluble fractions a base (m.p., 180° C.) was obtained. It proved to be identical with adlumine, the greater portion of which was present in the mixture of bases not removed by chloroform from acid solution (see above).

The less soluble fractions yielded a pale yellow base, the melting point of which was raised only slowly from 192–194 to 194–196° C.

For the purpose of further purification it was dissolved in chloroform-methanol, rendered just acid with concentrated hydrochloric acid, and the solution repeatedly evaporated with chloroform to remove other solvents. A slight turbidity was removed with the aid of charcoal and the colorless filtrate evaporated to a small volume. On cooling, colorless plates of a hydrochloride rapidly separated. Ethyl acetate was added to facilitate manipulation

and filtration. The hydrochloride as thus obtained melted at 259° C. and no depression was observed when it was admixed with a specimen of bicuculline hydrochloride from *Dicentra cucullaria* (3). Yield, 4 gm.

The base regenerated from the hydrochloride was recrystallized from chloroform-methanol and melted at 196° C. When however the supersaturated solution was seeded with a crystal of bicuculline melting at 177° C. the same form was obtained, and no depression in melting point was observed when the two were mixed. Oxidation with dilute nitric acid yielded hydrastine.

Isolation of Adlumidine and Adlumine

The chloroform extract (AC) was evaporated to a small volume, filtered with the aid of charcoal and evaporated to a thin syrup. In the course of several days a crop of colorless sparingly soluble crystals had separated. Examination under a lens and subsequent recrystallization proved this alkaloid to be homogeneous.

It was filtered off, washed first with chloroform-methanol and then with methanol. It was dissolved in a large volume of chloroform, in which it is sparingly soluble, an equal volume of hot methanol added and a slight turbidity removed by filtration with the aid of charcoal. The colorless filtrate was rapidly evaporated. While still hot, colorless fine stout prisms separated. After cooling the base was filtered off, washed first with hot methanol, then with a little chloroform, and again with methanol. After drying, the ***adlumidine*** as thus obtained melted sharply at 235° C. to an orange-colored liquid without visible decomposition. Recrystallization changed neither the appearance nor the melting point. Yield, 5 gm. Calcd. for $C_{19}H_{15}O_6N$; C, 64.59; H, 4.25; N, 3.97%. Mol. wt., 353. Calcd. for $C_{19}H_{17}O_6N$; C, 64.23; H, 4.79; N, 3.94%. Found: C, 64.98, 65.14; H, 4.79, 4.75; N, 3.86, 3.92%. Mol. wt., 297, 308 (Rast). Methoxyl, negative.

The filtrate from the crude adlumidine by a fortunate chance was allowed to remain overnight, during which time large almost colorless rhombic plates separated. This product was filtered off, washed with cold methanol and recrystallized from chloroform-methanol. Fine rhombic plates melting sharply at 180° C. were thus obtained. Yield, 6 gm. This proved to be identical with the ***adlumine*** previously isolated from the chloroform extract (C). Calcd. for $C_{21}H_{21}O_6N$; C, 65.80; H, 5.48; N, 3.66; 2 OMe, 16.15%. Found: C, 66.02; H, 5.53; N, 3.88; OMe, 15.71% (mean of duplicates).

Isolation of Protopine

The mother liquor from the adlumine was freed of organic solvents and the residue dissolved in dilute hydrochloric acid. A slight turbidity was removed with the aid of charcoal, and the cooled filtrate treated with excess potassium hydroxide. After several days the granular base was filtered off, washed with water and dried. It was recrystallized by solution in a mixture

of chloroform and methanol and evaporating the clarified filtrate (charcoal) to a small volume. When a crystal of protopine was added to the hot solution a copious crop of this alkaloid rapidly crystallized out. Yield, 7 gm.

There are two crystal forms of protopine analogous to the two forms of allocryptopine. The more common, but less stable, form consists of warty aggregates of excessively minute needles, which melt at 205-206° C. The more stable form melts at 211° C. when pure and consists of large stout highly refracting prisms with many faces developed. It is much less soluble in chloroform and a mixture of the two may be easily separated by means of this solvent. when, as frequently happens, they crystallize side by side. When slowly heated a mixture of the two forms melts at 210-211° C.

The specimen of protopine obtained from *Adlumia fungosa* melted at 211° C. either alone or admixed with a specimen prepared for purposes of comparison from *Dicentra spectabilis*. Calcd. for $C_{20}H_{19}O_5N$; C, 67.99; H, 5.38; N, 3.97%. Found: C, 67.81; H, 5.45; N, 4.02% (mean of duplicates). The alkaline filtrate from the protopine was extracted with ether which removed a little protopine. It was then saturated with carbon dioxide and the whole thoroughly extracted again with ether. There was thus obtained about four grams of an alkaloid which proved to be adlumine.

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STUDY OF A FILTER-PASSING STRAIN OF A STREPTOCOCCUS ISOLATED FROM A CASE OF BOVINE MASTITIS[1]

By Frances H. Prissick[2]

Abstract

A strain of green streptococcus, No. 403, obtained from a case of bovine mastitis and similar to that usually associated with chronic mastitis in cattle, has under stated conditions been passed through Berkefeld N and Chamberland L 3, L 5 filters. It has been recovered from the filtrates and proved to be identical in its biological characteristics with the original unfiltered strain No. 403.

Filtrates of this organism grown in K medium have given the largest number of recoveries, these being 18 out of 20, or 90%. Filtrates of growth in nutrient broth were recovered in 1 out of 20 filtrations, or 5%.

Observations on the cultural and morphological appearance of the organism during the stages of its recovery are made. The interpretation of these experiments in the light of Zinsser's postulates is discussed.

During the past few years a large school of bacteriologists has grown up, which has substituted the theory of a monomorphic life of the bacterium for that of a metamorphic one. The conclusions reached by investigators from their experiments are well reviewed by Evans (1) and Hadley, Delves and Klimek (4). Only those results which are directly pertinent to the subject of this paper will be mentioned in any detail.

In 1926, Ramsine (12) reported the discovery of a growth in culture media which were being incubated for sterility during the process of making Dick toxin. This growth was first observed after an incubation period of three or four days in dextrose bouillon. It appeared as a light sediment or flocculence, which staining showed to consist of Gram negative mycelium, containing Gram positive granules. Further culture and incubation gave rise to forms which resembled streptococci in morphological appearance, and which later developed into definite cocci in chains.

Hauduroy (5) claimed to have filtered various kinds of bacteria through new filter candles, and to have recovered these organisms in their recognized form, after numerous passages on lactose litmus agar. Later, Hauduroy and Lesbre (6) described a technique for the cultivation of hemolytic streptococci from the filtrates of their growth in peptone water. The type of filter candle used in these filtrations is not mentioned. The organism appearing in these cultures corresponded closely in its morphology to that noted by Ramsine (12). Out of 26 strains of streptococci filtered, 10 produced filter-passing forms. This work was later confirmed by Urbain (15).

Dealing principally with the members of the dysentery group, Hadley, Delves and Klimek (4) were able to dissociate this bacillus into R, S, and G colonies by means of incubation in bouillon containing pancreatin or lithium chloride; the G type proved to be filter-passing.

[1] *Manuscript received February 1, 1933.*
Contribution from the Animal Diseases Research Institute, Hull, P.Q. This paper is a preliminary report.

[2] *Research Assistant, Animal Diseases Research Institute, Hull, P.Q.*

In 1931, Kendall (10) advocated the use of a new medium as a means of dissociating bacteria and as a producer of filter-passing forms. This medium is made from intestine, preferably that of swine. Other tissue such as heart, brain, spleen or kidney of various animals has been used, but has proved less satisfactory than that of hog intestine. The finished medium is said to contain protein of a naturalness closely approximating that of the animal body, and to be almost free from protein degradation products. Kendall (9) has claimed to have passed several types of streptococcus, *B. typhosus*, *H. influenzae*, *Staphylococcus aureus*, and Noguchi's *Leptospira icteroides* through Berkefeld N filters, after growth has taken place in his K medium. He also suggests its use in the cultivation of the filterable viruses.

At the time of writing this paper, very few workers have come forward with results obtained in the use of this medium. Among these are Hoffstadt and Youmans (7) who have used K medium as a means of dissociating a strain of *Staphylococcus aureus* into R, S, and G type colonies; it was effective in culturing the G forms.

Rivers (13) found K medium unsuitable for the cultivation of vaccine virus.

Zinsser (17) has stressed the necessity for postulates in dealing with the problem of filterability and a bacterial life cycle. The need for such postulates is great, because of the confusion and misunderstanding existing amongst workers on this subject, due chiefly to the propounding of theories unaccompanied by adequate proof.

The bearing of these postulates on this work will be discussed at the conclusion of the following preliminary report of experiments with K medium upon the possible filter-passing properties of a strain of green streptococcus.

Streptococcus. (Strain No. 403)

Viridans Group, Type α

Source

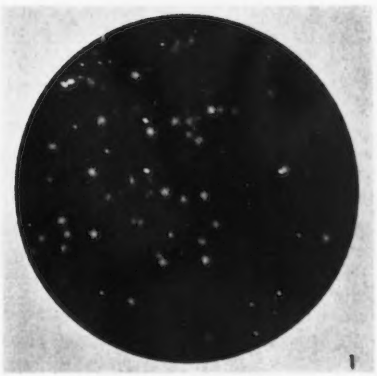
The streptococcus used in these filtration experiments was isolated by Dr. Rosell of Oka from the milk of a cow with chronic mastitis, and is of the type usually associated with that disease.

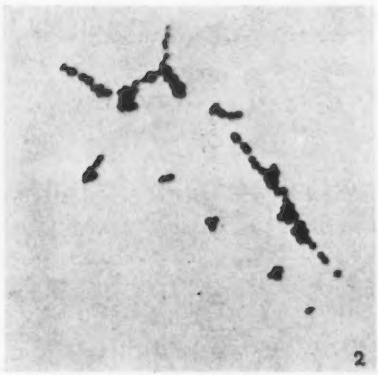
Colony

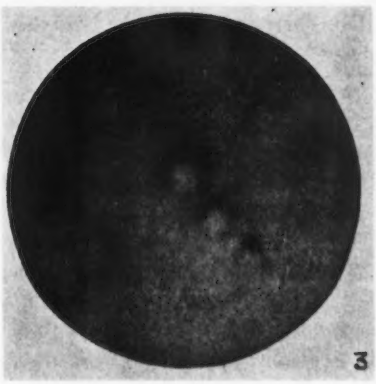
This is fairly small, measuring from 0.25 to 0.5 mm. in diameter, flat, opaque, smooth with edges entire, and growing well on chocolate agar at 37° C., where a yellowish green ring of coloration is produced, in ratio to the colony 2 : 1 (Figs. 1 and 2).

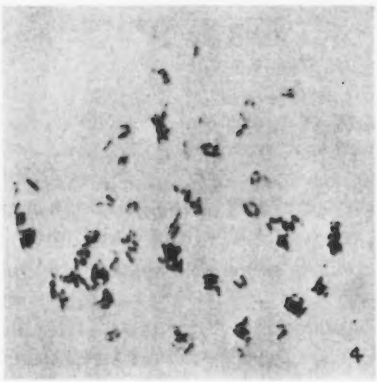
Morphology

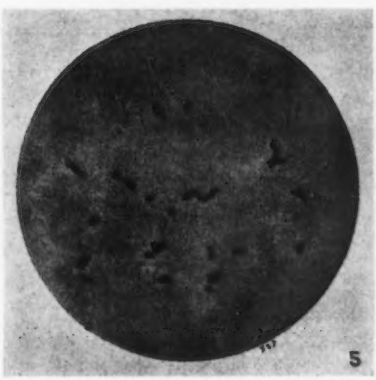
When grown on chocolate agar at 37° C. for 24 hr., the streptococcus measures from 0.8 μ to 0.9 μ in diameter, taking a deep Gram positive stain, and growing as single rather oval cocci, paired or in short chains. In lactose broth, chains of medium length are produced. No capsule has been demonstrated.











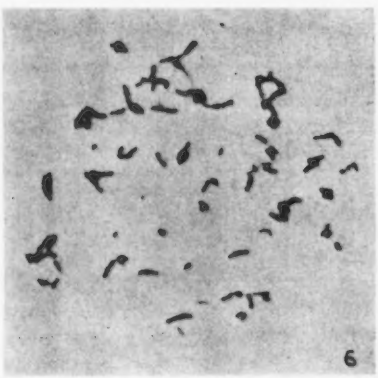


FIG. 1. *Colonies of Streptococcus 403 on chocolate agar, 24 hr. at 37° C.* × *4.* FIG. 2. *Streptococcus from the same type of colony; Gram's stain.* × *1300.* FIG. 3. *Type A colony, "Stage 1".* × *15.* FIGS. 4 and 5. *Type of organisms found in "Stage 1" colonies.* (FIG. 4. *Methylene blue.* × *1300.* FIG. 5. *Gram's stain.* × *1300.*) FIG. 6. *Type of organisms found in older "Stage 1" colonies; methylene blue.* × *1300.*

Biochemical

Sugar reactions were read after five days' incubation at 37° C. in Hiss' serum water, made with 1% of each sugar and 1% of Andrade's indicator.

Lactose	Mannite	Salicin
Acid	—	—
Coagulation (slight)	—	—

Pathogenicity

Rabbits remained unaffected by a dose of strain No. 403 grown 24 hr. on blood agar, re-suspended in 15 cc. of normal saline, and from 5 cc. to 10 cc. injected intravenously.

The inoculation of 1 cc. of a 24-hour-old culture on chocolate agar, re-suspended in 10 cc. of normal saline, into the peritoneal cavity of a mouse, leads to no noticeable clinical signs or symptoms.

Technique

Below are given the details pertaining to the preparation of the principal media and the filtration apparatus as used in all the experiments reported.

Media

K medium

1. The small intestine of a hog was washed, chopped fine and extracted with four volumes of 95% alcohol for 48 hr. at 37° C. This extraction was repeated twice, making three extractions in all.

2. The alcohol was removed and the tissue extracted once again with two volumes of benzol, until the tissue looked brown and clear.

3. The tissue was dried in warm air, and ground as fine as coffee grounds in a ball grinder or very fine meat chopper.

4. Two per cent of the dried tissue was added to 1000 cc. of Tyrode solution, for which either of the formulas in Table I proved to be satisfactory.

TABLE I

Formula No.	NaCl, gm.	KCl, gm.	$CaCl_2$, gm.	$MgCl_2$, gm.	NaH_2PO_4, gm.	$NaHCO_3$, gm.	Dextrose, gm.	Aqua dist., cc.
1	8.0	0.2	0.2	0.01	0.05	0.2	0.8	1000
2	7.0	0.2	0.2	0.1	0.05	1.0	1.0	1000

5. The solution was heated in flowing steam for 25 min. and the sediment allowed to settle.

6. The pH was then adjusted with sodium bicarbonate to 7.4–7.5.

7. The supernatant fluid was decanted and filtered through a Berkefeld N filter candle, and retitrated, if found necessary. The medium was then distributed into tubes in 15-cc. and 10-cc. amounts.

8. After sterilization in the autoclave for 20 min. at 15 lb. the tubes were incubated 48 hr. for sterility.

The final pH was 7.3 to 7.4.

Agar

In culturing the filtrate, two agar bases were used, to the first of which was added 3% of fresh sterile ox-serum, and to the second the same percentage of fresh defibrinated ox-blood. The agar was melted in the autoclave and cooled to 45° C., the sterile serum or blood mixed thoroughly with each, and petri dishes poured. The plates were incubated 48 hr. at 37° C. for sterility. Both kinds of agar were used in all transfers from the cultures of the filtrates of growth in K medium and nutrient bouillon. For the sake of convenience, these have been termed respectively agar No. 1 and No. 2.

Agar base No. 1. To 1000 cc. of beef infusion are added: 0.5% of sodium chloride, 2.0% of proteose peptone (Difco), 1.5% of dried intestine, 0.8% of dextrose (c.p.). The mixture is heated for 45 min. at 70° C. and titrated with sodium bicarbonate to pH 7.5. Agar (3%) is added to this and melted in the autoclave. The fluid is titrated and distributed into flasks which are sterilized for 25 min. at 15 lb. and then incubated two days for sterility. The final pH should be 7.4.

Agar base No. 2. This medium is a very slightly modified form of Gray's broth (3) for culturing streptococci.

Fresh beef heart, freed from fat and fibre, is ground in a chopper and infused in the ice box overnight, in the proportion of 500 gm. of meat to 500 cc. of tap water.

In the morning this is heated to 20–25° C. and strained through a single layer of flannel. The filtrate is boiled for one hour, filtered through filter paper and made up to volume. The following are added: 1.5% of proteose peptone (Difco), 0.5% of sodium chloride, 1.0% of lactose (c.p.), and 1.0% of gelatin. This is placed in flowing steam for one hour, filtered through filter paper and titrated to pH 7.8–7.9. Agar (2.5%) is added and the whole is steamed until it melts, after which it is distributed into sterile flasks and autoclaved for 30 min. at 10 lb. These are incubated 48 hr. at 37° C. for contamination.

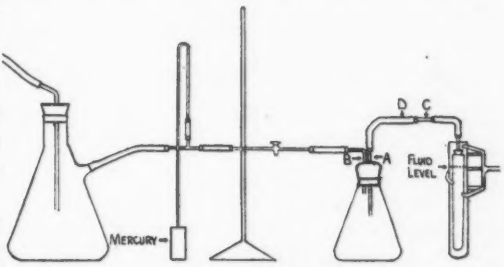
The chocolate agar used in the study of some of the subcultures is that of Crowe.

Apparatus

The accompanying illustration shows the set-up of the filtration apparatus.

The filter flask is plugged with a rubber cork, through which run glass tubes, *A* and *B*, and the cork and neck of the flask covered with tin foil. One flask with rubber tube, *D*, is attached to *A*, and the end wrapped in tin foil. Tube *B* is plugged with absorbent cotton. The whole flask with tubing attached is wrapped in paper and sterilized in the autoclave.

The filter candle is fitted with a rubber cork through which is passed narrow glass tubing. One end of this reaches down to the bottom of the candle and the other is fitted tightly with rubber tubing, which is joined to a short glass tube, *C*. The joints are fastened with wire. Tube *C* is plugged with absorbent cotton and wrapped in tin foil. The candle and its fittings are then wrapped in paper and sterilized in the autoclave.



Filtration apparatus.

The flask is unwrapped and the tin foil wrappings covered with boiling paraffin. Half of the glazed portion of the candle, the cork, and part of the rubber tubing attached, are also covered with several layers of boiling paraffin. Tube *B* is connected with the suction pump. Taking sterile precautions, tube *D* is quickly joined to *C*. The filter candle is inserted into the fluid to be filtered, and pressure applied slowly.

Filter Candles

Filtration took place through only new Berkefeld N filters, and through both new and used Chamberland L 3 and L 5 candles.

The Chamberland candles were cleaned by a method recommended by Dr. E. G. D. Murray of McGill University, Montreal. This method has proved to be most satisfactory, as it enables the filters to be used again many times without the clogging of pores and loss of speed.

This method of cleaning is as follows. Contaminated candles are washed through by gravity with about 200 cc. of distilled water and then soaked overnight or longer in 10% hydrochloric acid. They are then washed by running through by gravity not less than 500 cc. of distilled water.* The candles are dried in the air or incubator at 37° C. and soaked in a 10% solution of ammonium nitrate, for a sufficient length of time to saturate the pores. After being dried thoroughly in the air or incubator they are burned at a dull red in a furnace, cooled slowly, washed by gravity using distilled water, and then dried in the incubator and wrapped in grease-proof paper. The unglazed portion of the candles should not be touched with the hands after soaking in hydrochloric acid, as passage through the filter may be facilitated by the presence of grease or oil (8).

The candle was then fitted with rubber cork and glass tubing as described in the diagram of the apparatus for filtering, and autoclaved for one-half hour at 15 lb. One set of each grade of filter employed was reserved solely for use in these filtrations, so that used candles had only been in contact with the control organisms which will be mentioned, and Streptococcus No. 403.

**A large glass container holding 1500 cc. of distilled water is used. To the neck of this is attached a 2-in. piece of rubber tubing, which is fitted onto the candle. The whole head is not more than 4 in. long.*

Experimental

Twenty filtration experiments on the possible filter-passing properties of this strain of Streptococcus No. 403 have been completed, the procedure following three main steps.

1. Incubation in K medium with control broth cultures.
2. Filtration through Berkefeld N or Chamberland L 3, L 5 candles.
3. Culture of the filtrates, with the purpose of recovering, if filter-passing, an organism with all the biological characteristics of the original strain No. 403.

All these experiments have been carefully charted, and as far as possible, all details considered essential to the filtration process recorded (11). One of these protocols is given as an example (see Table II).

I. *Growth of the Cultures Prior to Filtration*

The streptococcus was seeded into tubes containing 15 cc. of K medium and nutrient broth respectively, and incubated at 30° C. Uninoculated tubes were incubated with these as controls. The length of time for incubating the cultures depends upon the rapidity with which the organism forms a type of growth in K medium, which will be described. This is a fairly heavy even suspension with some precipitation and flocculence. It was found that 65–72 hr. is sufficient incubation time to afford a very definite change in the morphological appearance of most of the streptococci in the medium. On being stained, the growth shows surprisingly few cocci for the density of the suspension. The cocci are Gram positive; the remainder consists of Gram negative fragments and also particles of darker staining matter. These particles, of which only a few have been seen in one microscopic field, are extremely small, being scarcely visible under a magnification of 1800, and are without definite structure as far as has been seen. Dark field examination shows numerous intensely shining bodies, some of which correspond in size to the particles described above. As the uninoculated K medium also shows bright granules, it is difficult to determine what is organic or inorganic material.

Theoretically, if one considers the possibility of there being a filter-passing stage in the life of a bacterium, and that this stage borders on the line between visibility and invisibility, it is not improbable that some of these particles may be connected with an intermediate stage between a filter-passing and non-filter-passing form.

Growth in nutrient broth has the same appearance macroscopically, as that seen in K medium. Microscopically, however, in contrast to the K medium, it shows larger numbers of Gram positive cocci in pairs and chains.

II. *Filtration*

As Zinsser has pointed out, filtration through ordinary laboratory filters is an uncertain process. So much depends upon the fluid in which the organisms are suspended, as well as on other factors such as the electrical charge of organism and candle, pressure and possible absorption or inactivation during filtration.

TABLE II

FILTRATION EXPERIMENT No. 18. STRAIN—STREPTOCOCCUS No. 403

Medium used	Incubation temp., ° C.	Time, hr.	Growth	Dilution of cultures	Control
"K" medium (made with Tyrode solution No. 2) pH 7.4	30	70	Good growth, even suspension with some flocculence	10 cc. of culture + 20 cc. of freshly sterilized physiological saline	18-hr. culture of *B. prodigiosus* on nutrient agar, washed off in fresh, sterile, normal saline; four drops of this emulsion added to the diluted culture before applying pressure
Nutrient broth (control) pH 7.4	30	70	Good growth, even suspension with some flocculence	10 cc. of culture + 20 cc. of freshly sterilized physiological saline	18-hr. culture of *B. prodigiosus* on nutrient agar, washed off in fresh, sterile, normal saline; four drops of this emulsion added to the diluted culture before applying pressure

Filtration temperature	pH of fluid filtered	Filter	Pressure	Time, min.	Amount filtered, cc.	Distribution of filtrate
Room temperature 22° C.	pH 6.8	Used (once) Chamberland L5	4½–5″ of mercury	15	Approximately 25–30	0.5 cc. to 10 cc. K medium 0.25 cc. to 10 cc. K medium 0.1 cc. to 10 cc. K medium 0.5 cc. to 10 cc. nutrient broth 0.25 cc. to 10 cc. nutrient broth 0.1 cc. to 10 cc. nutrient broth 1 drop to agars No. 1 and 2
Room temperature 22° C.	pH 6.8	Used (once) Chamberland L5	4½–5″ of mercury	14	Approximately 25–30	0.5 cc. to 10 cc. K medium 0.25 cc. to 10 cc. K medium 0.1 cc. to 10 cc. K medium 0.5 cc. to 10 cc. nutrient broth 0.25 cc. to 10 cc. nutrient broth 0.1 cc. to 10 cc. nutrient broth 1 drop to agars No. 1 and 2

Recognizing the influence exerted by any or all of these or other factors, 20 filtrations were performed under the following conditions:

A. Preparation of the cultures. To every 10 cc. of culture to be filtered, 20 cc. of freshly sterilized physiological saline was added, and the pH of the dilution noted. It was thought possible that an acid or alkaline pH might influence the passage or non-passage of the organism through the filter, so the pH was made to vary from 6.0–7.6, as may be seen from the chart which gives a summary of these experiments and their results. In the first nine filtrations, the pH of the K medium culture on filtering was acid or very slightly alkaline, with the pH of the control broth quite definitely alkaline. In the last series, the pH of both K and broth cultures was the same on filtering. The results suggest that the passage or non-passage of the streptococcus through the filter was not influenced by the pH.

B. Process of Filtering

(1) The apparatus was set up as illustrated, with sterile precautions.

(2) As a filter control, an 18-hr. culture on nutrient agar of either *Staphylococcus albus* or *B. prodigiosus* was washed off in freshly sterilized physiological saline, and about four drops of the emulsion added to the culture to be filtered immediately before the pressure was applied.

The *Staphylococcus albus* strain was isolated from milk, and measured from 0.3 μ to 0.9 μ in diameter, the average being 0.6 μ.

In later filtrations, the Staphylococcus was replaced by a culture of *B. prodigiosus* obtained through the kindness of Dr. T. M. Rivers of the Rockefeller Institute for Medical Research, and which had been used extensively as a filter control. Both organisms grew well on all media employed in these experiments.

(3) Transplants were made from the diluted cultures before filtering in order to be certain of their viability.

(4) Reduced pressure was applied very slowly, increasing until the manometer registered not more than 6 in. of mercury, usually remaining at about 5 in. The time taken to filter approximately 25–30 cc. varied according to the pressure, and grade of the candle, but was never longer than 20 min.

Filtration always took place at room temperature.

III. *Culture Technique Following Filtration*

The filtrates of the cultures in both K medium and nutrient broth were removed by means of a graduated pipette with a capillary point which reached through tube *A* to the bottom of the flask, and distributed in 0.5-, 0.25- and 0.1-cc. amounts into tubes containing 10 cc. of K medium, and nutrient or dextrose broth. These were incubated at 30° C. In addition, one drop of the filtrate was planted on agar Nos. 1 and 2, and incubated along with the other cultures. In all experiments where petri dishes were used for agar, uninoculated plates were incubated as controls, as were also uninoculated tubes of both K medium and nutrient broth. It may be suggested that

contamination could take place during the processes of putting together and dismantling the filtration apparatus. All possible sterile precautions were taken, and on no occasion was a filtrate found to produce growth other than such as will be described.

Description of Primary Growth

As other investigators have reported, Hauduroy (5, 6), Hadley *et al.* (4) and Ramsine (12), the filtrate growth in its early stage did not resemble that of the original strain of organism, either culturally or morphologically.

In the 20 experiments performed with this strain, No. 403, any growth that was obtained showed first on the agar plates, or in tubes of K medium receiving the filtrate of growth in K medium. Occasionally, and much later, growth occurred in nutrient broth tubes which had been planted with K medium filtrate. Only once has an organism been recovered from a broth filtrate, and that had been incubated in K medium.

The primary growth was difficult to classify. After 48 hr. at the earliest, usually several days however, a very slight sediment could be seen at the bottom of the tube. This sometimes developed into a flocculence. The structure of this sediment and flocculence was indefinite, the main body of it being Gram negative with a few particles which stained faintly Gram positive. Like those found in the growth in K medium before filtering, these particles were also without definite structure, though inclined to be spherical; they might be either organic or inorganic matter. This flocculence has been transferred from tube to tube of K medium, and from the first transplant an organism was recovered. This organism resembled the diphtheroid to be described shortly.

After 48 hr. incubation, or as soon as the sediment appeared in the tube, transplants were made to agar Nos. 1 and 2 and incubated at 30° C. These transplants were made by removing a few drops of fluid containing some of the sediment, by means of a sterile Pasteur pipette, and streaking one drop on agar Nos. 1 and 2. If no growth appeared in 72 hr. the line made by the inoculating loop was scraped and resown on fresh agar, which was incubated again at the same temperature. In addition, fresh agar plates were planted from those tubes of K medium and nutrient broth which had received the K and broth filtrates. This process was repeated until growth in the form of colonies became visible. These took from a few days to almost two weeks to show. No plate culture was ever discarded before two weeks time, unless the agar had become too dry to afford any likelihood of growth.

Recognizing the errors which might be caused by contamination, the following precautions were taken.

When any transplant was being made from tube or petri dish, one of the control plates was exposed to the air for the same length of time, and streaked with the medium from the uninoculated K medium and nutrient

broth tubes. It was not considered necessary to filter these control-tube contents previous to plating out, owing to the fact that the final sterilization of the media was by autoclaving and not by filtration. The only growth ever obtained on these control plates was fungus, and an occasional colony of *Staphylococcus albus*. Nothing suggestive of the growth from cultures of K filtrates appeared. It was thought that the dangers of exposing the plates frequently to the air might be overcome by using Petroff's culture flasks. This was not found to be satisfactory, however, as the water of condensation made it impossible to see the very small colonies on their appearance; though these flasks were used successfully in later transplants, as many as six to eight serial transfers being made in the same flask. Incubation of cultures under reduced oxygen pressure gave no more successful results than did incubation under aerobic conditions.

Development of the Colonies

The first colonies developing on agar from the K filtrate, which had been incubated in K medium or broth, were always similar. For convenience sake, the streptococcus colony as found in the stock No. 403 culture has been called type T colony, and the form recovered in the filtrate growth, type A colony.

The following four main "stages" in the development of the A colony from its first appearance on agar until it regained the T form have been found to occur.

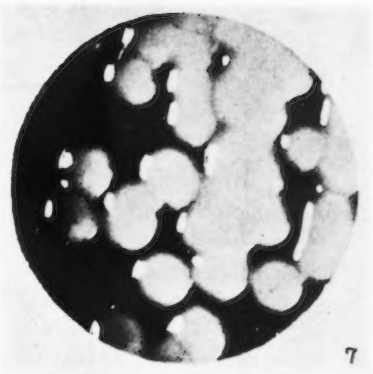
1. The colony was minute, rather glistening, smooth and slightly opaque; and after 48 hr. the centre was seen to be finely granular. These colonies grew with difficulty, it being rare to obtain growth from transplants of them before 48 hr. After a few subcultures, however, they reproduced more rapidly on either agar 1 or 2 at 30° C. (Fig. 3.)

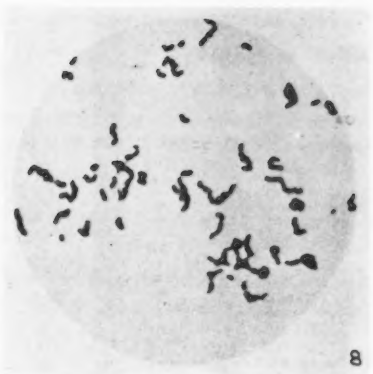
The organism from these colonies was a Gram positive diphtheroid-like rod, slightly curved at times; occasionally coccoid forms could be noted. (Figs. 4, 5, 6). These colonies remained stable in form for several generations, and the organism found in them did not undergo any very noticeable change beyond a slight lengthening. They grew poorly, if at all, in broth.

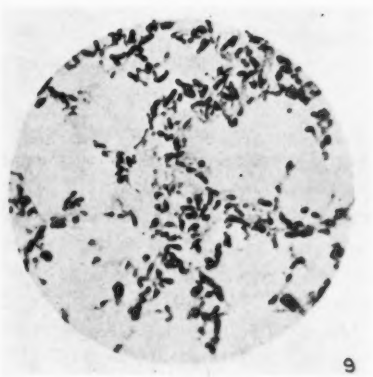
2. The second stage came about gradually. The diameter increased and the colony became more opaque and acquired a slight greenish white pigmentation (Fig. 7). Growth, which was mucoid on smearing, was more luxuriant on agar No. 2, and also in broth.

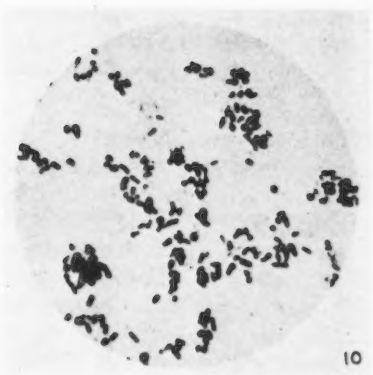
The organisms were found to be still diphtheroid-like, but stained more deeply (Fig. 8). Club forms appeared in broth cultures. This stage grew equally well at temperatures of 30° C. and 37° C.

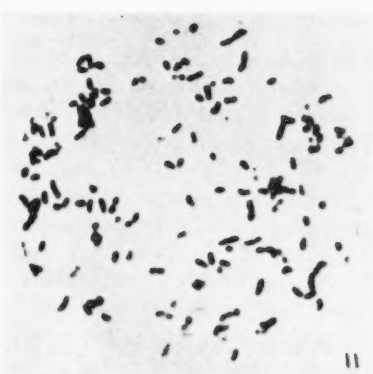
As the cultures aged the colony increased in size, with edges slightly undulate, and the organisms displayed a tendency to stain unevenly with Gram's. Pigmentation became definitely greenish, and the smears revealed the bacillary forms shortening to become coccoid, yet retaining the outline











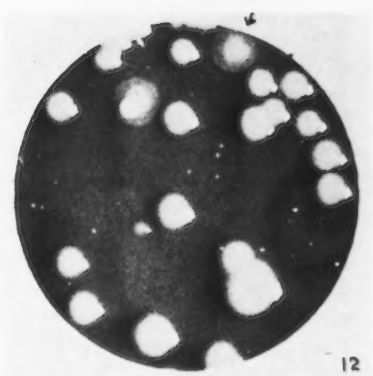


FIG. 7. *Typical "Stage 2" colonies.* × *10.* FIGS. 8–11. *Types of organisms found in "Stage 2" colonies; Gram's stain.* × *1300.* FIG. 12. *Typical "Stage 3" colonies.* × *10. (Notice two "Stage 4" colonies appearing.)*

of their diphtheroid phase. The short rods and cocci stained deeply Gram positive, while the shadowy forms were Gram negative. Granules which took the Gram stain could be seen, some within the diphtheroid-like organisms, and some appearing to be free. (Figs. 9, 10, 11). After this stage, cultures were incubated at 37° C.

3. In the third stage, the colony was pea green in color, with little or no coloration on chocolate agar, and measured 0.5–1.5 mm. in diameter. (Fig. 12).

Coccoid forms predominated throughout the smears, a few short, thickened diphtheroids being found. (Fig. 13).

It has been said that from only one broth filtrate sown in K medium were streptococci recovered. These grew first as "stage 3" colonies after 35 days incubation at 30° C. and contained cocci in pairs and short chains. After transplanting four or five times they gave rise to colonies typical of strain No. 403. There was no diphtheroid phase found.

4. The pea green color of the colonies gradually became fainter and the diameter decreased until it measured about 1 mm. The centre was slightly raised and surrounded by a smooth ring. There was a slight green coloration on chocolate agar. (Fig. 14).

The organisms were cocci, usually larger than those of the original No. 403 strain, and occurred for the most part singly, paired and in very short chains. (Figs. 15, 16). A few remaining shadowy Gram negative forms could be seen. By means of four or five serial transplants to tubes of blood or chocolate agar and incubation at 37° C., a streptococcus was recovered which was found to be biologically similar in every way to the unfiltered strain No. 403. Occasionally it happened that at first the recovered streptococcus was inclined to produce slight acidity in salicin, but after one or two transfers it reverted to the same reaction as before filtration. Tested for virulence by the method used for strain No. 403, the recovered streptococcus proved to be non-pathogenic. This streptococcus has been re-filtered and recovered from filtrates of K medium, several times.

Above is described the series of "stages" which have been obtained in culturing the filtrates in most of the 20 experiments. In several of these, the colony development did not include the third and fourth stages, but the coccoid forms appeared in the whitish green type of growth. After seeding in nutrient, or Gray's broth (3), and re-transfer to chocolate agar or agar No. 2 at least ten times, a streptococcus identical biologically with strain No. 403 was recovered. It has sometimes been found that the diphtheroid, if left on agar No. 2 at 37° C. for six or seven days or longer, may change more quickly into the form of a coccus than it would when transplanted often to fresh medium. The transition from the short coccoid and diphtheroid organisms as illustrated in the photographs to cocci in chains may take place quite quickly. When transferred to blood agar No. 2 at 37° C. these forms have

been seen to develop into chains in four to five hours. As two of the photographs show, the streptococci could be seen appearing in the midst of the Gram positive and Gram negative diphtheroid and coccoid forms. (Figs. 17 and 18).

Study of the Diphtheroid

A belief that the diphtheroid, as found in the first visible growth on agar after filtration, was not the primary form of organism, led to the following study.

A series of cultures was made from a tube of K medium which had received 0.25 cc. of a filtrate of Streptococcus No. 403 grown in K medium, and filtered through a Chamberland L 3 candle. This culture tube had been incubated five days at 30° C. when a flocculence appeared. The structure of this flocculence was identical with that already described.

From this flocculence, transplants were made to a series of petri dishes containing agar No. 2, and to slides on which had been mounted thin blocks of the same agar. These were incubated at 30° C., and one of each examined every 40 min. for 42 hr. The smears from the cultures in petri dishes gave the most information. Examination was made under an 1800 magnification.

In three hours a few granules staining very deeply with a 1 : 10 dilution of carbol fuchsin, and intensely blue with Giemsa, were seen. With Loeffler's methylene blue, they resembled the metachromatic granules found in the *Corynebacteria*.

In four hours time, these granules had elongated. When stained, the original granular structure was still deeply colored, while the remainder took a lighter shade. Throughout the following 18 hr. the elongation continued, until the organism showed as a short, slightly curved, barred or granulated rod. (Fig. 4). There remained only a few of the deeper staining granules. In 48 hr. the colonies described as A type were visible to the naked eye.

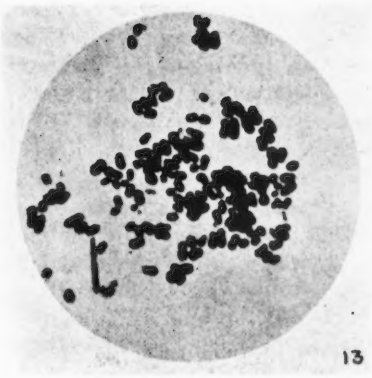
The diphtheroid organism differs from the Streptococcus No. 403 in its sugar reactions, which are as follows:

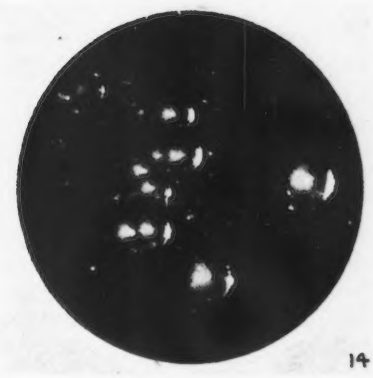
	Lactose	Mannite	Salicin
Acid	—	—	slight
Coagulation	—	—	—

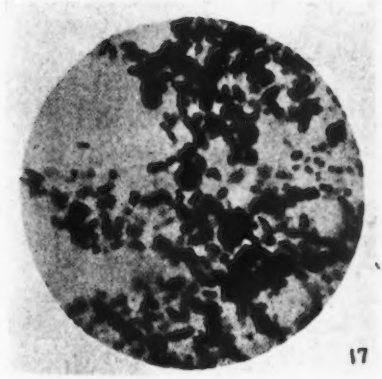
From the mucoid nature of the growth of the diphtheroid in its early stages, it would seem reasonable to expect the presence of a capsule. No capsule has been seen.

The diphtheroid organism was non-pathogenic when 1 cc. of a 24-hour-old culture on chocolate agar suspended in 10 cc. of sterile normal saline was inoculated into the peritoneal cavity of a mouse, as was also 1 cc. of the filtrate of a four days' growth of Streptococcus No. 403 in K medium when inoculated in a similar manner.

Inoculation of: (1) the stock, unfiltered Streptococcus No. 403; (2) the filtered and recovered Streptococcus No. 403; (3) the diphtheroid organism







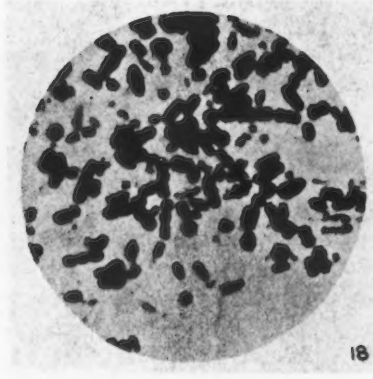


FIG. 13. *Type of organism found in "Stage 3" colonies; Gram's stain.* × *1380.* FIG. 14. *Typical "Stage 4" colonies.* × *10.* FIGS. 15 and 16. *Type of organism found in "Stage 4" colonies.* (FIG. 15. *Gram's stain.* × *1360.* FIG. 16. *Carbol fuchsin, 1 : 10.* × *1380.*) FIGS. 17 and 18. *Smear from "Stage 4" colonies growing eight hours on nutrient blood agar.* (FIG. 17. *Giemsa.* × *1460.* FIG. 18. *Gram's.* × *1460.*)

recovered from filtrates; and (4) filtrate of Streptococcus No. 403 grown in K medium, was made into the udders of two cows. From clinical evidence it seems that in the great majority of cases, infection from one quarter of the udder is most unlikely to pass into the other quarter of the opposite side. That is, the right front quarter will not become infected from the left rear quarter, whereas the left front probably will in time. This is thought to be due mainly to the fact that the udder is divided into two halves by a double septum of fibrous tissue. Therefore the same cow was able to receive two different inoculations. In making these inoculations it was not expected that there would be any acute attack of mastitis, the disease being chronic in nature. Nor was it expected that there would be any changes involving the tissues, probably until after the next lactation period. But it was hoped that streptococci of the strain inoculated, not present already in the milk, would be recovered from it. However, there is not enough known about the mode of infection of the udder in chronic mastitis to justify the placing of much importance upon the results of the following experiments.

With all possible sterile precautions, milk was taken from the four quarters of the udder of two cows and plated out on chocolate agar. Forty-eight hours incubation at 37° C. produced only a few bacterial colonies, none of which were streptococci. Brom thymol blue tests were run on each quarter and proved negative.

1. In the first cow, inoculation was made into the teat canal of the opposite quarters, *i.e.*, the right front and left rear. Each received 10 cc. of an emulsion in sterile normal saline, of a 24-hr. growth on chocolate agar of Streptococcus No. 403.

The right front was inoculated with the stock unfiltered strain, and the left rear with the filtered and recovered strain.

Every 48 hr. milk was taken from all four quarters and plated out on chocolate agar. In five days, the two quarters which had been inoculated with the streptococcus showed a light green reaction with Brom thymol blue. In ten days time from the date of inoculation, streptococci identical with strain No. 403 were found growing on the plates planted from the milk of the right front and left rear quarters of the udder.

2. The second cow was inoculated by the same technique as was described above. An emulsion in 10 cc. of sterile normal saline was made from a 24-hour-old growth on chocolate agar of the diphtheroid-like organism recovered from filtrates of strain No. 403 in K medium, and inoculated into the left rear quarter of the udder. The right front quarter was inoculated with 10 cc. of the filtrate of a 72-hr. growth of Streptococcus No. 403 in K medium. The filter used was a Chamberland L 3.

In 11 days the culture from the milk of the left rear quarter showed streptococci, and in 14 days the cultured milk from the right front quarter also showed cocci in short chains. These proved biologically identical with strain No. 403.

Discussion

In all, 20 filtration experiments on the filter-passing properties of a strain of Streptococcus known as No. 403 have been completed. This organism was obtained from a case of chronic bovine mastitis. Out of this number 18 (90%) of the cultures in K medium have been filtered under the stated conditions, and under those conditions a streptococcus identical in every way with the unfiltered strain has been recovered.

From the same number of nutrient broth cultures filtered under the same conditions, only once has the original strain No. 403 been recovered. This recovery was obtained from a dilution of broth filtrate planted in K medium.

Steck (14) has discussed the possibility of there being a relationship between the diphtheroids and streptococci found to be present together or separately in the diseased or healthy udder of cows. He mentions Klimmer's suggestion that there might be a transition of diphtheroids into streptococci, and also refers to Diernohofer's finding of diphtheroid forms in cultures of streptococci. Steck has studied these diphtheroids and streptococci of the udder, and found them to be biologically different, though he considers that there may be some relationship between them during the course of the disease.

Culturally and morphologically, these diphtheroids are almost identical with the diphtheroid organisms which have been described in this paper.

Recently a report has been made by Evans (2) of forms of streptococci appearing in material and cultures from cases of epidemic encephalitis. She describes spore-bearing rod forms, some of which resemble the diphtheroid-like organisms found in cultures from the filtrates of Streptococcus No. 403 grown in K medium, though no bodies similar to those termed "gonidia" have been seen on the walls of the diphtheroids. The nearest approach to the formation of such gonidial structures might be found in the place wherein the diphtheroid forms partially lose their Gram positive properties, and appear granular, or as a Gram negative sheath surrounding a Gram positive coccus. This is illustrated in Fig. 9 where these Gram positive granules may be seen in the shortened forms.

Evans (2) concludes with this statement, "Life cycles are a law of nature; . . . . Algae, fungi and protozoa—the plant and animal groups standing next higher than the bacteria—exhibit marvellous life cycles. It is unreasonable to think that a law of nature which becomes more and more complex with the descent in the scale of life, would be suspended in its lowest known forms."

By means of motion microphotography, a recent study of *B. shigae* growing on various types of media has been contributed by Wyckoff (16). Using K medium and medium containing lithium chloride, the various "life cycle forms" were found to occur. Wyckoff does not consider that there is evidence in his work of stages in a life cycle, or that the "quick reversions" indicate the presence of filterable forms. He cannot yet offer an explanation of the "slower reversion".

TABLE III

SUMMARY OF FILTRATION EXPERIMENTS

Experiment no.	Medium used	pH of fluid filtered	Filter candle	Time between filtration and first growth sign, days	Time between filtration and final recovery, days	Medium from which organism was recovered	Final result
1	K medium 1*	6.2	New Berkefeld "N"	7		Organism died out	−
	Broth	7.6	New Berkefeld "N"				−
2	K medium 1	6.7	New Berkefeld "N"	3	24	K filtrate in K medium	+
	Broth	7.6	New Berkefeld "N"				−
3	K medium 1	7.1	New Berkefeld "N"	3	25	K filtrate in K medium	+
	Broth	7.6	New Berkefeld "N"				−
4	K medium 1	6.0	New Chamberland L 3	4	32	K filtrate in K medium	+
	Broth	7.6	New Chamberland L 3				−
5	K medium 1	6.3	Used Chamberland L 3	4	31	K filtrate in K medium	+
	Broth	7.2	Used Chamberland L 3				−
6	K medium 2	6.8	New Chamberland L 5	6	30	K filtrate in K medium	+
	Broth	7.4	New Chamberland L 5				−
7	K medium 2	7.0	New Chamberland L 5	7	35	K filtrate in K medium	+
	Broth	7.5	New Chamberland L 5				−
8	K medium 2	7.2	Used Chamberland L 5				−
	Broth	7.4	Used Chamberland L 5				−
9	K medium 2	7.4	Used Chamberland L 5	5	33	K filtrate in K medium	+
	Broth	7.5	Used Chamberland L 5				−
10	K medium 2	6.4	New Chamberland L 3	5	32	K filt.: in "K" and broth	+
	Broth	6.4	New Chamberland L 3				−
11	K medium 2	6.6	New Chamberland L 3	4	34	K filtrate in K medium	+
	Broth	6.6	New Chamberland L 3				−
12	K medium 2	6.8	Used Chamberland L 3	5	33	K filtrate in K medium	+
	Broth	6.8	Used Chamberland L 3	35	39	Broth filt.: in K medium	+
13	K medium 2	7.0	Used Chamberland L 3	4	35	K filt.: in "K" and broth	+
	Broth	7.0	Used Chamberland L 3				−
14	K medium 2	7.2	Used Chamberland L 3	3	32	K filtrate in K medium	+
	Broth	7.2	Used Chamberland L 3				−
15	K medium 2	7.4	New Chamberland L 3	5	35	K filtrate in K medium	+
	Broth	7.4	New Chamberland L 3				−
16	K medium 2	6.4	New Chamberland L 5	6	34	K filtrate in K medium	+
	Broth	6.4	New Chamberland L 5				−
17	K medium 2	6.6	Used Chamberland L 5	5	36	K filtrate in K medium	+
	Broth	6.6	Used Chamberland L 5				−
18	K medium 2	6.8	Used Chamberland L 5	7	31	K filtrate in K medium	+
	Broth	6.8	Used Chamberland L 5				−
19	K medium 2	7.0	Used Chamberland L 5	6	35	K filtrate in K medium	+
	Broth	7.0	Used Chamberland L 5				−
20	K medium 2	7.2	Used Chamberland L 5	3	24	K filtrate in K medium	+
	Broth	7.2	Used Chamberland L 5				−

**Indicates the number of the Tyrode solution used in the K medium.*

The intention is now to quote each of the four postulates set up by Zinsser and see if they have been in any way fulfilled by the experiments performed with Streptococcus No. 403.

I. "It must be possible to filter a suspension of a well-defined pure culture of bacteria through a filter which holds back the characteristic forms; and these original forms should not appear in the filtrate or in cultures made with adequate amounts of the filtrate on suitable media after incubation of at least two or three weeks."

The requirements of the first half of this postulate would appear to have been met. Of course, as has been noted elsewhere, the suspension fluid, *i.e.*, K medium diluted with physiological saline, may have proved more favorable to the passage of organisms than nutrient broth diluted in a like manner. This being so, it would be fairly reasonable to expect that the control, *B. prodigiosus*, should pass through the filter pores at least occasionally. This was never found to be the case. The second half of the postulate seems also to have been covered. Filtrates were never found to contain characteristic streptococci. They contained no organisms at all, that could be identified. The amounts of filtrate planted seem adequate, and the media, blood and serum agar, also suitable; the original streptococcic forms were not recovered for at least two weeks, usually longer, and then after having passed through a diphtheroid phase.

II. "There must be evidence of growth of some kind in cultures made of this filtrate, but without evidence of the presence of the original normal forms."

The flocculence occurring in tubes inoculated with filtrate is taken as evidence of growth, inasmuch as organisms were recovered only from such tubes, and these organisms did not resemble streptococci in their morphology.

III. "The culture of the filterable or minute form must be carried in a series of several generations."

This postulate cannot be considered as fulfilled. The flocculence was carried through only two generations and then planted on blood agar. From this transfer streptococci were eventually recovered after passing through the aforementioned diphtheroid-like phase.

To the requirements of this postulate might be added the further test of a second filtration. That is, the filtrate culture containing flocculence should be re-filtered, and the second filtrate distributed as was the first. The attempt could then be made to recover the organism in its original form from the cultures of this second filtrate.

IV. "It must be possible to recover the normal forms from such successive 'filterable attributes'."

In the light of the third postulate this fourth one has only been partially fulfilled. The normal forms of streptococci have been recovered from filtrates of growth in K medium, but not after successive passages of the filtrate

cultures from tube to tube. The suggestion may be offered that the slow recovery is probably after all only latent growth. This may be so; but in that case it would seem more reasonable to expect the normal cocci to appear and reproduce, than to find a diphtheroid phase giving place slowly to coccoid forms.

In conclusion, it may be said that no consideration whatever is given here to the suggestion of some workers that ultramicroscopic viruses may be identified with "filterable forms" of bacteria. Nor is it maintained that these filter-passing forms of a strain of streptococcus form a filterable stage of a bacterial life cycle, though the possibility is not excluded. Such evidence as there is seems to point to the presence of a viable particle in the filtrates of growth in K medium. The nature of this particle has not yet been explained satisfactorily. Whether it be a filterable phase, an altered or a degenerated condition brought about by some action of the medium upon the bacterial cell, we do not know. At the present time there do not seem to be sufficient facts offered and confirmed by investigators of the problem, to warrant any definite conclusion as to the existence or non-existence of a regular bacterial life cycle including a filterable phase.

Acknowledgments

The writer's thanks are due to Dr. E. A. Watson and Dr. C. A. Mitchell for their interest and advice; to Dr. L. J. Rhea and Dr. E. G. D. Murray of Montreal, for their criticism of the manuscript; and to Mr. H. L. Boyd of this Institute for his trouble in making the accompanying photographs.

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THE STRUCTURE AND BEHAVIOR OF UTRICULARIA PURPUREA[1]

By Francis E. Lloyd[2]

Abstract

This paper presents an account of the more important structural features of a representative of a New World group of species within the generally accepted genus *Utricularia*, namely, *U. purpurea*. These species have been relegated to the genus *Vesiculina* by Barnhart, and the evidence here presented supports his views.

A general description is given of the habit and habitat of the plant, in which also the particular points of its form and structure are set forth. The important features of the internal anatomy are given.

The structure and behavior of the trap are described. It is shown that, while the mechanical working of the trap is in some respects strikingly different from that of the other species outside *Vesiculina*, the same fundamental plan of structure is adhered to. A detailed account of the structure of the door and of the threshold and their manner of action is given.

Utricularia purpurea is the sole representative in eastern North America of an exclusively* neo-tropical group of floating, or loosely anchored, submersed plants, in this having much the habit of *U. vulgaris*. Kamienski, in his analysis published in Engler and Prantl, includes the species under the section *Lentibularia*, associating with them *U. vulgaris* and some similar forms, and separating these from the *Megacista* (Sect. VIII) on the one hand and from the *Parcifolia* (Sect. X) on the other. This segregation and association, when viewed in the light of knowledge of the structure of the bladders (traps, as I prefer to call them), appear highly unnatural, however useful they may be in practical taxonomy. Barnhart (1) refers the *U. purpurea* segregate to *Vesiculina* Raf. on other grounds than the structure of the bladders—a confirmation of my conviction that, if any segregate of plants now inclosed in the genus *Utricularia* should be separated, certainly *U. purpurea* and its associates deserve this distinction. My own opinion is based chiefly on a study of the traps and, in addition to the immediate purpose of this paper, I shall try to show the evidence for my contention. For the rest, I propose to afford a more intimate account of the structure and behavior of the trap in *U. purpurea*, at the moment altogether lacking from our records.

The material used was collected in a small lake eight miles west of St. Jerome, Quebec, where my friend, Professor Marie-Victorin, had found it years previously. The name of the lake, Lac à la barbotte, serves but meagrely to identify it among the hundred others of the same name.

The plant is apparently not entirely freely floating, but is more or less anchored in the muddy bottom—possibly because of the sinking of shoots (especially winter buds) in the fall to settle in the mud. Viewed in the mass,

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* *Barnhart (1) tentatively included F. Mueller's U. tubulata of Australia under Vesiculina Raf. Through the courtesy of Dr. A. B. Rendle, I have been able to examine material of Mueller's original (and only) collection, and can state quite definitely that his plant has not the characters of the purpurea type and it is highly probable that it conforms to the vulgaris type*

it is an unattractive plant, looking more dead than alive, brown and muddy in color. It is only on finding the ends of the shoots to be supplied with spreading verticils of branches each bearing a trap at the end, when young beautifully circinate and pale green in color, that one realizes that the plant is alive. The older parts of the shoots are purplish in color and devoid of traps, they having been shed by abscission. Add the fact that the plant is clothed with numerous glandular trichomes secreting mucilage to which stick all manner of minute forms and debris, and the rusty, ill-conditioned look is explained. It grows in shallow water, forming a zone of sorts just at the edge of the water-lily zone. It seems to flower in shallower water where it can mass more abundantly.

Previous studies of the traps of this or related species have been made by Goebel (7), Luetzelburg (15), Dean (4), and myself (12). Goebel described the curious trichomes found in a group issuing from a knobby projection near the middle point of the door in *U. purpurea;* Luetzelburg confirmed this for a South American species which he called *U. elephas*, because of its prominent trunk-like curved rostrum extending from the ventral side of the entrance. Thereto he added notes on the shape and structure of the door (of *U. purpurea*) indicating the forwardly flexed rim of the free door edge, but inadequately. He described also the threshold and front surface of the door of *U. elephas*. Dean, as a zoologist, was more concerned with the kind of food caught, but incidentally described quite briefly the trichomes of *U. purpurea*. He pronounced against the idea that the action of the trap is spasmodic, thinking that the door acts as a passive valve under which small animals crawl. The door is so weak as to allow such animals "to fall in of their own weight", he avers, all of which we shall see to be wrong. His interpretation of the functions of the valve hairs—namely, those of the door—were made to harmonize with his views of the passive character of the door. For my own part I have examined previously a species similar to, if not identical with, *U. elephas* Luetz., sent to me in preserved condition by Dr. Hoene, of Sao Paulo, Brazil. From it I was able to see clearly those structures together with the positional relations thereof, which enabled me to compare them directly with the corresponding ones in such species as *U. vulgaris*, *U. gibba*, etc. As my observations have been confirmed and amplified by study of living material of *U. purpurea*, I need not recount them here. I may say, however, that the details of structure and function are so different from those of *U. gibba*, etc., that a separate and full treatment is justified.

Form of the Plant

The plant consists of an axis of rather uniform thickness (about 1 mm. in diameter), tapering very slightly towards the apex. At intervals arise verticils of secondary axes (four to six in the verticil), these in turn verticillate, but becoming dorsiventral by the suppression of some of the tertiary axes. The axes of the younger verticils are all strongly circinate, and each bears at its end a young trap enwrapped within the roll. There are no "leaves". The

apparent radial symmetry of the plant is betrayed by the structure of the central cylinder which, as will be seen, is bilaterally symmetrical, as also by the evident bilateral symmetry of the winter bud. The purplish hue, less pronounced at the growing ends, is due to anthocyanin contained in the sap of the inner cortical cells and in the inner of the two courses of cells in the walls of the trap.

The winter buds (turions), by no means as highly specialized as those of *U. vulgaris*, are made up of verticils of compactly crowded circinate branches, separated at short distances by slightly developed internodes. The branches all bend upwards from the morphological lower side of the system.

The axes are dimorphic—one sort being widely spreading with shorter internodes, the other having longer internodes and much shorter branches.

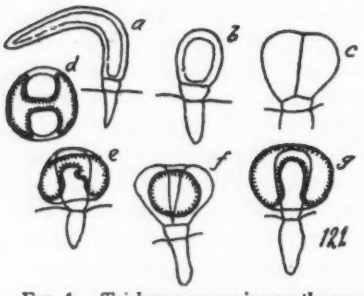

FIG. 1. *Trichomes occurring on the surface of U. purpurea: a, sickle-shaped trichome, always bent downwards (backwards); b, oval mucilage secreting trichome; c, oil secreting trichome; d-g, various aspects of this trichome with the oil lying between the secreting capital cells and their common cuticle.*

The epidermis is crowded with three-celled trichomes of three kinds, a unique situation among the Utricularieae. The most obvious is an elongated, sickle-shaped trichome which is very abundant, giving the surface a furry appearance (Fig. 1). Scattered among these is a second in much smaller numbers—a sessile trichome with an oval capital. Both have a thick-walled capital cell. The third kind is a low sessile trichome with a two-celled capital. The two cells eventually separate longitudinally but remain covered by their common cuticle, which forms a spherical investment and becomes filled with a colorless fatty oil. Lodged between the cuticle and the two cells which secrete it, the oil takes a curious form, due of course to the shape of the cavity and to the fact that the walls are not wetted by the oil. I have extracted this oil with ether and proved it to be fatty. It has no odor, or at least not distinguishable from the general odor of water plants. The ether extract takes up the chlorophyll in solution. These three trichomes are morphologically a unit with the other forms found in and on the trap, consisting of a basal cell (epidermal) inserted between larger epidermal neighbors, a middle strongly cutinized small cell (mid-cell) and the frequently non-cutinized capital, of one or two cells according to sort. The whole surface of the plant is coated with mucilage produced by the trichomes; we may suppose by the two kinds, aside from the oil-secreting. All sorts of foreign matter adhere to the mucilage so that it presents a dirty appearance.

The trichomes are produced very quickly. Only the extreme tips of new axes are smooth. The earlier to develop are the oval mucilage and the oil trichomes, which appear to occupy nearly the whole surface; a little later the sickle-shaped trichomes make their appearance, on the outer flanks of the curves of circination first (Plate I–*2*).

Anatomy

The chief axis is cylindrical, gently tapering to the apex. The epidermis is composed of elongated cells, five times as long as broad. Between the ends, and usually at three-angled contacts, are inserted narrow fusiform cells, which are the basal cells of the three-celled trichomes, of which (as mentioned above) there are three kinds. There are no stomata. Under the epidermis there are three courses of parenchyma cells, constituting a cortex, which is broken up by large longitudinal air spaces (Plate I–*1*), each separated from its neighbor by radiating plates of a single layer of cells, penetrated intercellularly by lacunae permitting communication between the longitudinal air spaces, which are interrupted along the internodes by occasional oblique partitions. The air spaces thus formed are much larger than in *U. vulgaris*. The radial partitions are three to five cells deep. They abut on a starch sheath surrounding the vascular tract. In the starch sheath I can observe no Casparian spots, though they are evidently present in *U. vulgaris*, as Hovelacque recorded. The vascular tract has a diameter about one-fifth of the entire axis, and is composed chiefly of thin-walled, elongated elements with transverse walls, called "fibres primitive" by Hovelacque. In *U. vulgaris* these cells have much thickened walls. Whatever their mechanical virtues, they are the only mechanical elements present. The xylem may not be present at all—most frequently not. If present, it is in an eccentric position, marking the upper, ventral side of a very obscurely dorsiventral structure.* When present, it is a very poorly developed trachea with weak thickenings and very difficult to identify in transverse sections.. Very occasionally, one finds a short length of trachea in a node, though the central cylinder may be devoid of xylem. The phloem occurs in isolated strands, a periclinal series of which lies against the endodermis, while, within, a few others lie scattered without obvious plan. The peripheral strands lie alternately with the cells of the endodermis, in the extreme angle between which lies a single longitudinal series of parenchyma cells, conspicuous for their dense contents. These I find in other species examined (*U. vulgaris*, *U. cornuta*) and have not before been noted. Hovelacque does not mention them. In common with the other species of *Utricularia* thus far studied, in the vascular system the xylem and phloem are dissociated and without any constant relation to each other beyond the indication of dorsiventrality indicated by the eccentric position of the xylem. The xylem is quantitatively almost negligible, though this does not mean that there is no movement of water. The established fact that the walls of the trap can transfer water from the inside to the outside, as observed by Brocher (2), suggests that this may be going on everywhere in the plant, but it is a matter subject to investigation.

Each node and the internode above are separated physiologically by a layer of suberized cells crossing the parenchyma. This appears to be an abscission layer, but is not such in fact. No true abscission occurs at this point. The plant is gradually overtaken by acropetal decay, without separation of the internodes.

* *As in U. neottioides (Goebel, 8).*

Structure of the Trap

Each of the verticillate branches normally bears at its end a trap. Just below the trap the branch is suddenly narrowed into a slender stalk on which the trap swings quite freely. When the plant is moved about in the water, the traps are waved this way and that. Abscission takes place in this zone and thus the old branches are lacking traps. The continuity of the stalk of the trap and the branch is characteristic of the group and is to be seen in no other *Utricularia* (Plate I–*3*; II–*19*). Through the stalk the single vascular strand of the branch passes into the trap and extends along the dorsal edge as far as the upper lip of the entrance, where it ends abruptly. There is no vascular tissue along the ventral edge (Plate II–*26*).

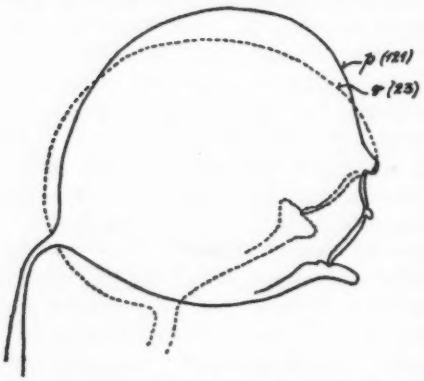

FIG. 2. *Silhouette of U. purpurea (p) superposed on that of U. vulgaris (v).*

The trap itself is of peculiar and elegant form, which is quite distinctive, best described as laterally compressed galeate. Its form is connected with the fact that the threshold lies parallel with the wall on which it stands, and is not rotated during growth as in other species. The dorsal marginal zone is arched over to meet the ventral zone to form the entrance. But the form can be understood only by means of a figure (Fig. 2; Plate I–*3*).

In color the trap is light green with a tinge of purple in the inner course of cells of the tissues especially in the region of the threshold. It is also exceptionally translucent, so that the interior structures may be more easily discerned than is usual in other groups. The surface is thickly clothed with the three kinds of trichomes mentioned above, the longest being curved backward, toward the stalk. Much mucilage is secreted and entangled by the trichomes, so that an abundance of detritus sticks to the surface.

When looking at the door from in front, one sees that the entrance is fairly oval, with a margin which lies in one plane, and this set somewhat

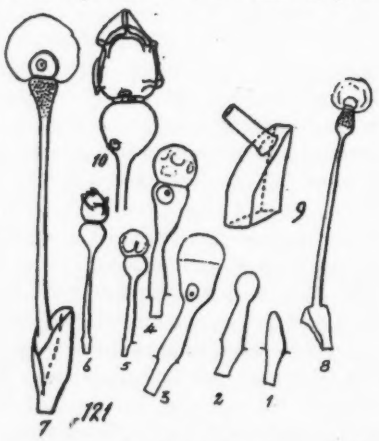

FIG. 3. *Developmental stages of the tubercle trichomes, in the order of the numbering. Nos. 1–4 on twice the scale of 5–8; 7, 8, the two kinds of trichomes in the mature state; 9, basal portion of the trichome, enlarged. Note that, in No. 6, the cuticle is expanding being raised above the surface of the capital cells in ridges; shown also in 10.*

obliquely with reference to a longitudinal axis, say one passing through the middle point of the entrance and the insertion of the stalk. Slight differences in position occur. The entrance is closed by the door and threshold, which lie somewhat within, leaving a free zone of approach clothed with short clavate trichomes, which project radially toward the middle point of the entrance (Plate I–*5*). Looking at the door itself, we observe at once a prominent tubercle occupying a position slightly above the middle point and bearing a radiating cluster of trichomes of two kinds (not one kind only, as has previously been supposed). The basal cell is long, slender and club-shaped. The walls near the top in the expanded portion are prominently pitted. This is surmounted by a very thin (Fig. 3), disk-shaped mid-cell, and this in turn supports a spherical gland cell with a ballooned cuticle. The gland cells contain a few chloroplasts. The two kinds of trichomes differ in the shape and proportions, as shown in the figures (Fig. 3; Plate I–*6*, *8*). Those with the smaller end cells occupy a more peripheral position. The tubercle in which these trichomes arise is composed of the expanded bases of the basal (stalk) cells, and is part of the outer course of the cells of the door, as will be made plain beyond.

These radiating trichomes are analogous to the tripping hairs so characteristic of the *vulgaris* type of trap (Lloyd, 12), but differ not only in form but in their lack of rigidity. They may be bent most easily, oftentimes without springing the trap. Because of their size and position, however, a small animal may scarcely approach the door without impinging on these hairs, and often the merest touch is sufficient to spring the trap, with the consequent capture of the animal. I shall enlarge on this point in due course.

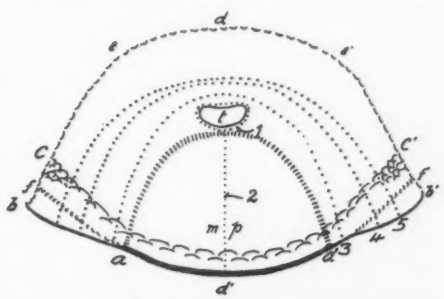

FIG. 4. *Diagram of door flattened out: a, a', middle reach of door edge heavily beaded; ab, a' b', lateral reaches, not beaded; b, inner angle coincides with the inner angle of the threshold; a f b, the lateral area of door surface in contact with the threshold; c c', position of the upper edge of the velum when the door is in position; a (t) a', middle piece of door; t, tubercle; b d b', edge of door attached to the walls of the trap; 1, line of flexure around the tubercle; 2, line of flexure along the axis of the middle peice; 3–5, successive lines of flexure during opening; e, e', points at which the door turns backward along the side walls of the trap.*

In order to examine critically the door, it must be dissected away. When lying in water it retains approximately its natural curvature, that of about a quarter-sphere. When flattened out it is nearly semicircular (Plate I–7), but departs from this form much more than does the door of *U. vulgaris*. This is related to the width of the threshold, which is narrow in *U. purpurea*, and to the position of the threshold, of which more later. For the sake of more ready description, the accompanying diagram is given (Fig. 4). The index letters mean as follows: *b a a' b'* is the lower free edge of the door which rests on the

threshold; $b\ d\ b'$ is the edge attached to the wall of the trap (the cells are therefore cut transversely along this line); $a\ a'$ is the middle reach of the door edge which is beaded and rests along the middle zone of the pavement epithelium of the threshold; $a\ b$ and $a'\ b'$ are the lateral reaches which traverse the pavement epithelium of the threshold to reach its inner angles; the zone marked off by $a\ b\ d\ t\ a'\ b'$ is the outer hinge zone; $a\ t\ a'$ is the middle piece of the door and the line 2 is a line of flexure along a longitudinal hinge; $e\ a\ b$ is a lateral area which turns back and is attached to the trap wall along $b\ e$; $f\ a\ b$ is a triangular area of the door which rests flat against the threshold, and not edgewise; $c\ c'$ indicates the position in which the velum lies against the door when in position of closure. 1 is a circular line of rotation of the tubercle, made possible because the tissue around and especially above the tubercle is thin (Plate II–*16*), obtaining a marked flexibility of the door in this region. The presence of this hinge-like region permits the rotation of the tubercle under impact on the trichomes sufficient to release the door edge from its emplacement, as we shall see.

Histology of the Door

We have adopted a terminology above to enable us to describe the histology of the door with relation to its movements.

It is composed of two courses of cells, continuations of the outer and inner epidermis of the trap wall, in this agreeing with the rest of the genus. It is also singularly translucent, if not quite as transparent as glass, so that, owing also to the thinness of the structure and the curvatures present, it is difficult to focus easily on either course of cells, especially the thinner ones. The walls of the other course shining through confuse the picture.

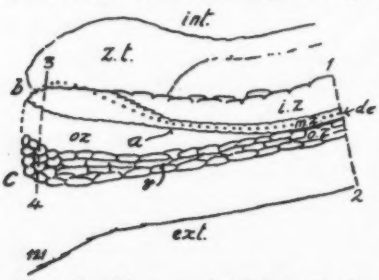

FIG. 5. *Diagram of one-half of the threshold. o z, m z, i z, outer, middle and inner zones; a b c, as in Fig. 4; z.t., zone of bifid trichomes. Section along 1–2, see Plate-fig. 22; along 3–4, see Plate-fig. 23. The position of the door edge when in position is shown by the heavily dotted line de to b; v, velum; int. and ext., interior and exterior of the trap.*

The best approach is to examine the door in sagittal section; the cut will then go through the tubercle (Figs. 7, 8; Plate I—*6*). This evidently separates two regions. The upper is, in section, in the form of a sigmoid flexure (Plate II–*14*). This is the outer hinge, but its action is more complicated than that of the outer hinge in the *vulgaris* type (Lloyd, 13). It will be seen that the two courses of cells alter their depth from the insertion above to the tubercle, the outer course of cells becoming thicker and the inner correspondingly thinner. The upper moiety of the hinge bends more readily outwardly, the lower inwardly; it is here that the maximum bending occurs when the door opens (Figs. 8–*A*, 8–*B*). If a median strip of the door is cut free in water it springs forward (Plate I–*8*, *9*), but when plasmolyzed

with 0.5 *N* potassium nitrate it returns only part way backward to its original position. This experiment demonstrates the region of maximum bending outwardly and inwardly. The tubercle is composed of enlarged cells of the outer course (Fig. 8; Plate II–*16*), while the juxtaposed inner course of cells is very thin. This, with the co-operation of a thin area (in the lower moiety of the door) around, but especially above, the tubercle, allows it a movement of rotation, very important in releasing the lower edge of the door from its firm emplacement. The thin region below the tubercle quickly passes over into the thicker portion to form a massive middle piece (Fig. 4), the cell courses of which are of equal thickness, except that the outer course becomes thinner toward the lateral regions where the door is attached to the wall (Plate I–*10*, *11*) and where maximum bending occurs with the opening of the door. This extensive middle piece is identified with a much smaller analogous region in the door of the *vulgaris* type (Lloyd, 13). The thin region surrounding the tubercle may be equated with the central hinge of that type. The doors of the two types, *purpurea* and *vulgaris*, are thus seen to be conformable fundamentally, though widely different in details of structure.

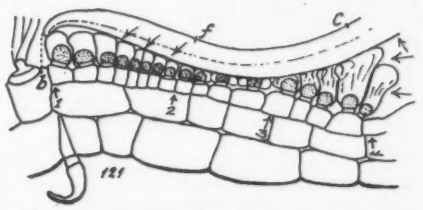

FIG. 6. *Section along 3–4, Fig. 5, shown diagrammatically: 1–2, the tilted surface of the pad area of the middle zone; 2–3, the area between the velum and pad near the end of the threshold; 3–4, the velum; c, f, b, as in Fig. 4; the arrow points below f indicate the direction of pressure of the door surface when the trap is set; those at the right, the water pressure.*

The free door edge is stiffened, but only throughout the width of the middle piece, by a forward-turning bead (Plate II–*15*). This fits into a groove (the middle zone) of the threshold when the trap is set. Its form, too, is important in relation to the velum, as will shortly be explained. The bead is made up of thickened cell walls of the door edge region (Plate I–*6*; II–*15*) (Luetzelburg, 15).

The large middle piece (Plate I–*11*) is seen to be uniformly thick except for a middle groove and composed of two courses of cells of equal depth. This part of the door, under the natural conditions of opening, does not bend sharply, but suffers first a shallow longitudinal folding, followed by a simple inversion of curvature from being bowed outwardly to inwardly. The maximum curvatures occur in the outer hinge above and below the tubercle, but more above, and around the corresponding lateral zones (Plate I–*10*). When the door is open the tubercle is therefore displaced inwardly (Fig. 7–*B*). The opening thus procured is circular, or nearly so (Fig. 7–*C*).

Having regarded the sagittal section, we are now enabled to study the aspect of the door as we face it from the outside, first *in situ*. Looking into the oval entrance (Plate I–*5*) we note at once the beautifully regular curvatures of the component cells. Above the tubercle (Plate I–*7*) they lie in paraboloidal curves, the more marked to the eye because of the intercellular spaces, more

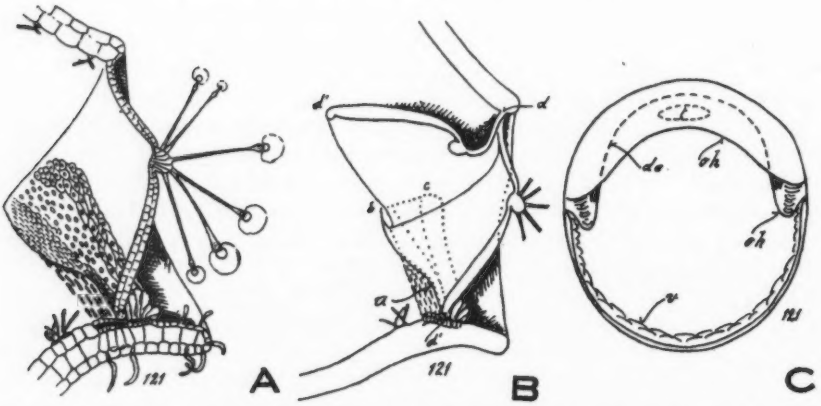

FIG. 7. *A, Diagram of entrance split sagitally (cf. Plate-fig. 6), the door in position when the trap is set. The door treated as if transparent, so as to show the position of the threshold. B, same, showing the position of the door closed and open; lettering as in Fig. 4; C, front view of entrance when the door is open; oh, folded edge of outer hinge; de, door edge; v, velum.*

or less gas filled, which signalize the rows of cells. The whole upper part of the door partakes of this character. Below the tubercle the pattern is continued by the curving rows of cells starting at the midline, where the cells are isodiametric, and passing downward and then upward, the cells now elongated in the direction of the curves of arrangement (Plate I–*12*, *13*; II–*25*).

In harmony with the above described behavior, the character of the cells is the following. Regarding the outer course of cells first, we note that in these, in the regions of maximum bending, the lateral walls are markedly zigzag and are supported by many broad rods placed in the radial walls, chiefly at their angles (Plate I –*13*; Fig. 9). The same features occur in the outer course of cells of the door in the *vulgaris* type, but in *purpurea* are even more striking in their development (Lloyd, 13). This kind of cell is confined to the central hinge area, where it is nearly isodiametric. As the central hinge area merges into the outer hinge, the walls run in more strictly radial fashion, are less zigzag, and the rods

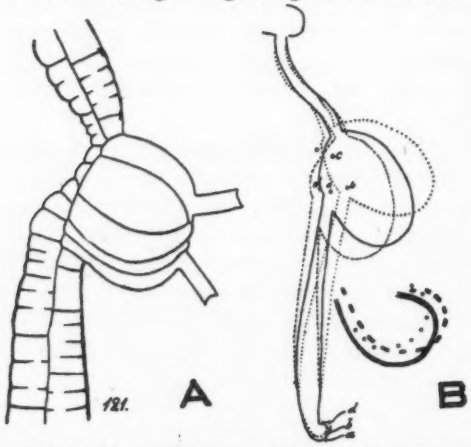

FIG. 8. *A, sagittal section of door in the region of the tubercle. B, model to show the effect of movements of the tubercle on the position of the door edge; a, closed position; b, d, any other position on either side of the door in normal position.*

are less conspicuous. In the middle piece and extending laterally from this, the outer course cells are elongated in the direction of the curves of arrangement and agree in form almost exactly with the underlying cells of the inner course. Their radial walls are supported by numerous but small and inconspicuous rods, but display almost nothing of the zigzag form (Plate II–*25*).

The inner course cells are elongated in the direction of the curves of structure; only in the middle line of the middle piece are they isodiametric, conformably with the cells of the outer course. The walls are somewhat thicker than those of the outer course, and again are supported by numerous rods, which, however, are usually round and break up the walls into bays. The direction of these walls changes from bay to bay, running a very slightly indirect course. In the midline of the middle piece, the rods of the there isodiametric cells have large strongly developed rods correlated with a maximum bending capacity along this line (Plate II–*25*). The outer walls, forming the inner door surface, are corrugated; the corrugations running roughly parallel to the line of attachment of the door to the trap wall are most pronounced and conspicuous in the outer hinge area (Plate I–*12*). We have seen them in their greatest degree of development in the *vulgaris* type, in which the corrugations are very regular and deep, lending to the whole inner surface of the door the aspect of being marked off in concentric circles (Kruck, 10, Lloyd, 14). In *U. purpurea* however, they are so vaguely regular that no such appearance emerges—indeed can scarcely be observed at all, except near the line of attachment of the door (Plate I–*12*) where they display their maximum development, and are chiefly characteristic of the outer hinge, where, owing to the "bellows" structure (as Ekambaram has called it), they insure the maximum bending capacity of the outer walls. The particulars of structure for *U. vulgaris* have been fully described in the previous paper (Lloyd, 14). In that area above the tubercle where the door is bent in a sigmoid flexure, we find the maximum degree of regularity and development; where indeed the flexures of the door on its opening are pronounced.

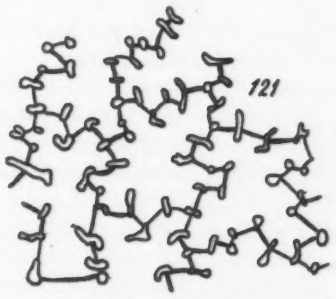

FIG. 9. *Cells of the outer course of the outer hinge of the door above the tubercle (cf. Plate-fig. 13).*

Briefly stated, the plan of the door structure is one with that of the *vulgaris* type, but, correlated with the position of the tubercle, which bears the tripping mechanism, the proportions of the various areas are different. The minute structure of the component cells is the same also qualitatively, the degree of development of rods and infolds or corrugations being correlated with the particular flexures of the door, which, after presently considering the structure of the threshold, we shall be in a position to elucidate.

The Threshold, its Anatomy and Function

(Plate II—*21–24*)

The threshold in *U. purpurea* and its close relatives (*U. elephas*, *cucullata*, etc.) differs in the remarkable feature that it lies parallel to the wall from which it springs and is scarcely raised. For the purpose of emphasizing this comparison I have inserted Fig. 2, in which the bare outlines of the two types, *vulgaris* and *purpurea*, are superposed. It is this feature which underlies the difference in form of the two kinds of trap, one which is not immediately apparent to the casual observer. The flatter ventral outline and deeply arched dorsal profile are related thereto and are the result of different developmental behaviors.

Viewed from a position on the axis of the trap, the threshold is seen to lie on the inside surface of the entrance to the trap, a short distance within, equal approximately to its own width (0.2 mm.). It is nearly semicircular (Fig. 7–*C*). The surface of the threshold is a band of glandular epithelium, forming a pavement, widening somewhat towards its ends (Figs. 5, 6). The pavement is composed of the capital cells of closely packed three-celled trichomes, which for other species, especially *U. vulgaris*, have been described by several observers, from Hovelacque on. All previous observers have supposed them to be alike, whereas there are in *U. purpurea*, as in *U. vulgaris* and other species, three zones, the outer, middle and inner, in which the characters and functions of the component trichomes are different (Plate II—*21–24*, *28*). The outer and middle zones are coterminous with the threshold; the inner zone is shorter and forms a lenticular patch lying between the inner zone and a zone of densely packed bifid trichomes (Plate II—*21–23*).

The outer zone is composed of capital cells with large swollen cuticles, and occupies a narrow band in the middle reach of the threshold spreading out fanwise toward the ends of the threshold. In this spreading region (*oz*, Fig. 5; between *2* and *3*, Fig. 6) an inner triangle has capital cells whose expanded outer membranes are scarcely if at all cuticularized (they are not appreciably stained with Sudan III) but are sufficiently bloated to occupy the whole area (Fig. 6), though the capital cells themselves appear very loosely scattered. In front of this and running along the whole front edge of the threshold is the velum, the capital cells of which have much ballooned and highly cuticularized membranes whose function is to heap up against the door edge when the trap is in set condition. Thus they block the entrance of water which would be especially prone to occur along the lateral reaches of the door edge where the triangular surfaces *a b f*, *a′ b′ f′* (Fig. 4) lie against the threshold owing to the door edge traversing it from point *a* to point *b* to reach the inner angle of the threshold at *b*, where the door edge articulates with the walls of the trap.

The middle zone is narrow along its middle reach (*m z* as far as *a*, Fig. 5), bowed backwardly, and toward each end expands into an elongate oval patch forming a pad tilted outwardly (between *1* and *2*, Fig. 6). The surface is of small, firmly tesselated capital cells. The narrow middle reach receives the

pressure of the door edge; the lateral pads offer resistance to the door surface included approximately in the triangles $a\,f\,b$, $a'\,f'\,b'$, Fig. 4. The tilting of the surfaces of the pads toward the outside permits the door to be cramped inwardly so that the door surface lies firmly against the threshold and comes into contact with the broad velum found in this region. The condition is illustrated in Fig. 6.

The inner zone is made up of capital cells with enlarged cuticles strongly cuticularized presenting a surface which is tilted outwardly, that is, in the same sense as the pads of the middle zone. Anatomically indeed these pads may be considered as a continuation of the inner zone, but I have preferred to designate these areas in terms of function. The inner zone has the function of resisting the inswing of the middle reach of the door edge. When the door opens the edge must glide over the inner zone.

The threshold therefore is seen to be a band of tissue the component cells of which are structurally adapted to hold the door firmly in position when the trap is set, and to make and keep it water-tight so that on exhaustion of water there is no inleakage.

The Action of the Door

Plate I—*5–9*; II—*14–17*.

Fig. 7 shows a sagittal section of the door, and Fig. 8, in greater detail, through the tubercle. This is composed of the hypertrophied bases of the trichomes, and is a much swollen area of the outer course of cells. Fig. 8–*B* is a diagram of a putative mechanical equivalent. Such a model can be only imperfect and no account can be taken of the delicate elasticity of the original. The point to be emphasized is that a slight rotation of the tubercle in any direction can have the effect of raising the lower edge of the door ever so slightly. This movement need not occur in precisely the middle of the door edge, as the model would indicate. Perhaps we should say that all that is required is a momentary distortion of the door edge. Such distortion will produce at the moment of occurrence an asymmetry to resistance of water pressure, this being all that is necessary. For, by virtue of the pressure of water on the door (owing to the reduced internal pressure—usually called negative pressure), the slightest asymmetry in position of the door edge allows the release of pressure against the ridge formed by the swollen cuticles of the inner threshold zone. The pavement epithelium, the door itself, and indeed every bit of surface of the trap is coated with mucilage which lubricates the parts so effectively that the contingent surfaces slide readily on each other. Once the shift of the door edge is accomplished, the water pressure swings the door in fully, or at least as fully as it is equal to (Fig. 7–*B*). This depends of course on the time which has been allowed for recovery from a springing. According to my own observations, it requires about two hours for the exhaustion of the water from the interior of the trap to the extent illustrated in Plate II–*19* and *26*—longer or shorter, according to individual traps. This is in contrast with *U. vulgaris*, which can exhaust itself sufficiently

for renewed action in a half-hour or less (Merl, Czaja). In *U. purpurea* the exhaustion proceeds till the sides of the trap become appressed together (Plate II–*26*). This is brought out most clearly in a trap in which on being tripped in air the bubble inclosed becomes distorted during exhaustion (Plate II–*19*).

It will now be appropriate to consider the nature and amount of disturbance of the tubercle trichomes which can cause sufficient distortion of the door edge to effect its release and so upset the "unstable equilibrium" (I quote Brocher), leading to the opening of the door. The following conclusions rest upon a very large number of experimental observations on fully developed but variously sized traps. The largest are about 3 mm. long; the small ones a half of this. They show, moreover, a very great difference of delicacy and this becomes more marked if in a culture there has been an accumulation of mucilage and adherent debris.

The ideal trap, if such an expression may be permitted, is one which responds most readily. This response is purely mechanical, as I have attempted to show in a previous paper (14). This species, *U. purpurea*, lends itself most strikingly to the elucidation of this question, for with care the trichomes of almost any trap may be disturbed by contact with a needle point without procuring a response. It is usual to be able to brush aside the trichomes repeatedly, provided direct impact is avoided. It is thus easy (much more so than in *U. vulgaris*) to prove that there is no reception or transmission of stimulus by the trichomes (as held by Kruck for *U. vulgaris*). As to the nature of the contact which is effective, one may say as follows. In a minority of cases the merest touch of a flat-sided needle point on the top of one trichome (so far as one could see with a binocular) at once released the mechanism. The delicacy is so great that one is usually surprised by the promptness of the response. This is the ideal condition. In other cases the trichomes may be displaced by a gentle upward, downward or lateral stroke of the needle, either without procuring response or with more or less prompt action. Frequently the action follows on the sudden resumption of the normal position by the trichomes after the needle has passed over them, or, briefly, on the back-swing. Or the needle point may be inserted between the trichomes and be pressed on the tubercle itself, with response. In others, response follows only on more vigorous impact. In common with *U. vulgaris*, lifting the plant out of the water results in the release of the mechanism in many traps by the water films accompanied by the ingulfing of air, as Brocher and others observed in *vulgaris*. The mere movement of the plant in the water often effects release, so that, because of the many traps present, often the small traps—young ones, yet unopened—are swallowed by large ones. In fact, a good way of experimentation is to use minute traps instead of a needle point. The more sensitive traps when put in action by the contact of a small trap (say 0.6 mm. long) promptly swallow it (Plate I–*4*). Now as the traps swing delicately on their pedicels, one gets an impression of extreme mechanical sensitivity. Obviously the impact of minute water animals (crustacea, worms, mites) suffices to actuate the traps.

The inswing of the door under pressure of the outside water is possible only because that organ is elastic, bending easily in every direction. Aside from the inherent physical properties of the cellulose of which its cell walls are composed, the resistance of the door to such water pressure arises from the fact that the door edge is a little longer than the threshold, that is, the line *b d' b'* (Fig. 4) is longer than the line *f d' f'* along the threshold. The result is that the door cannot swing in without buckling. This takes place along the middle line (Fig. 4–*2*) of the middle piece of the door, reaching from the door edge to the tubercle (Plate II–*25*). As soon as this release is accomplished, the middle piece as a whole becomes bowed in the sense opposite to that of the normal position, so that the flexure beginning in the midline is transferred to the outer hinge. This it follows just above the tubercle, this structure being bent inwards with the middle piece; it thus disappears from view in the profile of the open trap (Fig. 7–*B*). The path of flexure above the tubercle lies in the area where the inner course of cells is thinner than the outer course. At the consummation of the inward movement, the opening must be a nearly circular one (Fig. 7–*C*), since the entering column of water will act as a solid rod, except for the fold of the outer hinge above the tubercle. This seems always to cut off a chord of the arc. By measurement, however, a trap about 2 mm. long can receive a glass bead about 0.57 mm. in diameter, this slipping in with no impedance. The trichomes might seem to be such, but the turning inward of the tubercle inclines them all inwardly and they are easily brushed aside by an entering object, aided by their general mucilaginous character.

The rate at which the door action takes place is such that the eye cannot follow any details of the movement. By means of motion pictures taken at normal rates, we know that the entire movement takes place within one-sixteenth of a second. Within this brief period the door swings in fully (Fig. 7–*B*) and then out again to its original position (Fig. 7–*A*). It is the swiftest movement in the plant kingdom, excepting perhaps ciliary movement. Naturally, one cannot make statements about the flexures of the door except as one may study the movement under pressure, preferably of a spherical object, unless slow motion photography is used. I have succeeded in this only in another species as yet, namely *U. vulgaris*, on which I shall report elsewhere.

Development of the Trap

We turn finally to the development of the trap, with special regard to the features peculiar to the subgenus as evaluated here. The course of development for the trap of *Utricularia* in general, as typified by *U. vulgaris* and nearby species, was worked out by Meierhofer (16). In brief, an ascidium develops at the end of a lateral organ. The dorsal lip of the ascidium develops into the door and the ventral into the threshold and lower lip of the entrance. The point of articulation of the two lips is the point *b* (Fig. 4); *b d' b'* of the door edge at first coincides with the inner margin of the threshold but continued growth lengthens it so that it comes to fit the curved line, running obliquely along the threshold, as already described (*b* to *de*, Fig. 5).

In the species under consideration, the differentiation of the structures of the trap which are peculiar to it begins very early. From the beginning, the outer surface is thickly clothed with the three kinds of trichomes above described (Fig. 10). A fully developed large trap is 2.5 to 3 mm. in length; but by the time it has reached a length of 0.3 mm. all the structures are distinct (Fig. 11), though not in a definitive condition. Of particular interest is the differentiation of the door, including the trichomes and tubercle, and of the threshold. In a trap 0.1 mm. long the amount of differentiation is scarcely enough to distinguish it from other species; nevertheless, the form of the ventral lip betokens the absence of that rotation which distinguishes, *e.g.*, *U. vulgaris* (Meierhofer, 16, Fig. 40). The door still lacks the stigmata of the species. The trichomes of the outer surface are numerous; the oil-bearing few in number, the sickle still absent (Fig. 10).

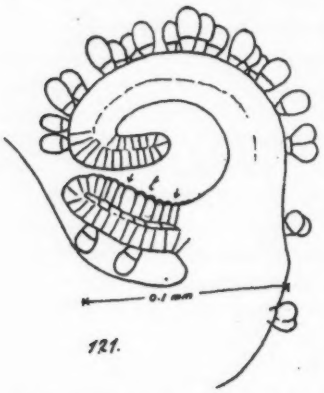

FIG. 10. *Sagittal section of trap 0.1 mm. long.*

A trap 0.3 mm. long. At this point in development the door projects inwardly and the lower free edge is shorter than the threshold. The trap is still tightly closed. The tubercle is scarcely visible, except that its cells have at this moment become short, cone-shaped projections. The curvatures of the door are distinct, but the cells undifferentiated, or but little so. The threshold is laid down, but no secondary differentiations marking the zones have appeared. The row of bifid absorbing trichomes backing the threshold (Plate II–*22*) is represented by a slightly raised cushion formed by the growth of the band of inner epidermis, just within but slightly separated from the threshold. From this point on, the changes are as follows.

The trichomes of the door tubercle (Fig. 3; Plate I—*5–9*; II—*18*, *20*, *27*). From a short, conical condition (Figs. 1, 3), in which the basal part is only very slightly enlarged, the protuberant portion elongates into a tubular tapering elongation furnished with a round knob, which later elongates and becomes pyriform, the slender end tapering into the stalk. A transverse wall now makes its appearance, after which both cells swell into nearly spherical moieties. A second transverse wall now is formed just above the first, cutting off the mid-cell—a longitudinally very thin cell, to which the cuticle remains adherent. The end or capital cell now enlarges to its maximum, forming a thick cuticle, and in shape ovoid (18 $\mu \times 25\ \mu$) (Plate II–*20*). The cellulose wall finally becomes globose leaving the large cuticle free. At first the cuticle wrinkles (Plate II–*27*), but subsequently expands and becomes filled with mucilage, forming (in *U. purpurea*) a large spherical bladder or balloon. This is the large trichome. The smaller stops short at the stage in which the swollen end is cylindrical, with two transverse walls (Plate II–*27*). The

capital cell has a less swollen cuticle. The outer end of the basal cells differs in shape also from that of the large trichome, as shown in Fig. 3. This region of the walls thickens and becomes pitted, affording an irregularly punctate picture (Plate II–*18*). At the latter stage of development the basal cell elongates fully, and the capital cell with its surrounding loose cuticle swells to its maximum. The tubercle is thus composed of the conjoined basal fractions of the basal cells of the door trichomes, which are part of the outer course of cells.

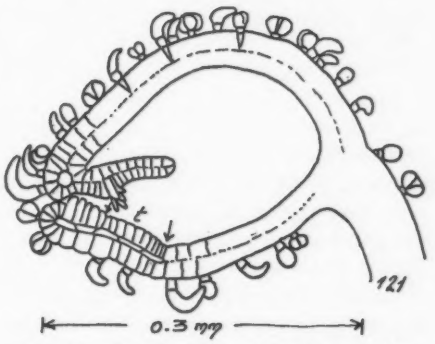

FIG. 11. *Sagittal section of trap 0.3 mm. long. The tubercle trichomes just appearing.*

The enlargement of the cuticles of the capital cells is worthy of remark. We already know that the cuticles of the outer zone of the threshold which furnish the velum arise by secondary expansion, forming relatively enormous balloons. To a less extent the same occurs in the cells of the inner zone, remaining (in *U. purpurea*) intact and forming an impedance to the inswing of the door. It appears to occur also in the oil glands of *U. purpurea* (Fig. 1). That the membranes are cuticular has been demonstrated by the usual staining methods; they stain deeply with Sudan III and IV. In the velar membranes there is evident growth from the base, as shown by the presence of double membranes (Plate II–*28*) at the sides of the balloons. At the other extreme of behavior there is the entire abjection of the cuticle, as occurs in the bifid and quadrifid hairs and the external glands, and in many other special cases. The genus is, in fact, notable for the various behaviors in regard to the cuticle of the glandular trichomes.

The threshold. At the early stage studied (0.3 mm. long), the threshold is differentiated as a band of glandular cells with bacilliform capital cells, their longer axes running transversely with respect to the trap, longitudinally with respect to the threshold. Those of the outer zone become deeper and, at the time the tubercle trichomes form the first transverse wall, begin to throw up the velar membranes, which soon reach their full expansion (Plate II–*28*) before the door is ready to come into its definitive position with its free edge along the middle zone. This zone is a lineal depression occupying the middle of the threshold, and is lined by two or three rows of compact capital cells. The inner zone develops the swollen cuticles somewhat later.

During all this time the trap remains completely closed but by the time the tubercle trichomes approach their definitive condition (somewhat later than as seen in Plate I–*27*) the mouth of the trap begins to open and the door to swing outwardly into its final position. Because of the position of the door during the growth of the trichomes, these all point inwardly and, as seen

during the opening of the trap, they maintain the flexures for some time, straightening out only when the final stage of adjustment is reached, the trichomes then assuming the radiating positions of the mature condition.

Taxonomic Position of the Group of Species Exemplified in *U. Purpurea*

The characters of structure presented by *U. vulgaris* and *U. purpurea* stand in such sharp contrast that one can hardly suppress surprise that the segregation which has been in general use remains accepted to-day in spite of Barnhart's studies (1). In Kamienski's treatment, he puts into the section *Lentibularia*, Gesn., *U. flexuosa*, *U. neglecta*, *U. vulgaris*, *U. minor* and *U. intermedia*. In the section *Megacista* DC., *U. stellaris* and *U. inflata*, and in the section *Parcifolia*, *U. exoleta*, *U. gibba* and *U. obtusa*. In addition to these, there are two species, one of which, *U. neottioides*, Kamienski places in a fourth section, *Avesicaria*. The other *U. rigida* Benjamin, of tropical Africa, is described by Stapf as being "nearly allied to" *U. neottioides* of Brazil, and, according to the description, has no bladders (traps). The name of the section indicates an opinion now known to be false, since *U. neottioides* has traps (Luetzelburg, 15, Lloyd, 12) and it is probable that *U. rigida* has also, though at present there is no evidence on this point.

The studies of Barnhart have led him to regard the segregates recognized by him as distinct genera. While an insufficient knowledge of the group does not permit me to make any far-reaching criticisms, it seems perfectly clear to me that the segregate *Vesiculina* has fully as much, I think rather more, to justify it than the generally recognized genus, *Polypompholyx*. The whole plant body, including the traps, is so very distinctive, and some of the differences I have indicated above. Grouped with *U. purpurea* on these grounds are the South American species, such as *cucullata*, *elephas*, Luetz., *U. myriocista*. The traps of these are all of the same character structurally, only *elephas* having a superficial distinction in a proboscis-like projection from the lower dorsal edge of the entrance (Luetzelburg, 15, Lloyd, 12). The traps may be briefly characterized, for taxonomic purposes, as having a door with a nearly central knob bearing radiating trichomes with widely inflated capital cells. This separates them at once from all other Utricularieae. The other species included with *purpurea* by Kamienski fall into two groups. In one, the insertion of the door is at the outer margin of the entrance, as in *U. vulgaris*, *U. diploglossa*, *U. oligosperma*, *U. intermedia*, *U. stellaris*, *U. flexuosa* (cf. Goebel, 7, figure, 6); *U. mixta*, *U. geminiscapa* and others belong here. In the other, the upper edge of the door is set back by the development of an overhanging or projecting extension of the trap wall, so that the door is approached as through a vestibule, as in *U. gibba*. Of this type are *U. pallens*, *U. pumila*, *U. exoleta*, *U. neottioides*, *U. herzogii*, etc. It requires but little skill to make the distinctions as regards the trap structure if the traps are present, as, however, they usually are in these floating plants. The structure of the traps is, however, important in the classification of the other

species, many of which are represented in herbaria by material which is so scanty and frequently altogether lacking in these organs that we may well take to heart the remark of Stapf (18): "In fact, a really satisfactory classification of this genus will only be possible when living or carefully collected and preserved spirit material is at hand". This applies to all parts, which are so delicate that, once subjected to drying pressure, only a very inadequate idea of the original plant can be obtained. As all the species mentioned in the preceding paragraphs have been so collected, I am enabled to entertain some confidence in saying that Barnhart's recommendation to separate the *purpurea* species is vindicated.

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EXPLANATION OF PLATES

PLATE I

Fig. 1. *Transverse section of an axis.*

Fig. 2. *Whole preparation of end bud of growing tip, showing early development of oil and mucilage trichomes.*

Fig. 3. *Silhouette of living trap showing the position of the door when the trap is set.*

Fig. 4. *A trap which has swallowed a young trap.*

Fig. 5. *Front view of the entrance of a trap, the door in position.*

Fig. 6. *Sagittal view of entrance. In this the door is not in its true position since the entrance has spread (cf. Text-fig. 6).*

EXPLANATION OF PLATES—Concluded

PLATES—*Concluded*

FIG. 7. *Somewhat over a half of a door laid out flat, showing the disposition of the cells (cf. Text-fig. 4).*

FIG. 8. *A median narrow piece of the door of a living trap, showing the maximum flexure when lying in water.*

FIG. 9. *The same on treatment with 0.5 N potassium nitrate.*

FIGS. 10 AND 11. *Transverse sections through the middle piece (Fig. 11) and the outer hinge at the same level (Fig. 10). Note the relative thickness of the cell courses, and the slightly thinner middle line of the middle piece.*

FIG. 12. *Inner surface of the outer hinge near its attachment to the wall. Note the lines indicating the infolds of the outer walls of the cells.*

FIG. 13. *Portion of the outer hinge obliquely above the tubercle, showing the outer course cells (with zigzag walls and heavy rods) overlying the radially elongated cells of the inner course.*

PLATE II

FIG. 14. *Sagittal section of the outer hinge and tubercle of the door, showing the change in dimensions of the outer and inner cell courses.*

FIG. 15. *The door edge, sagittal section, showing the beading.*

FIG. 16. *Sagittal section of the tubercle, showing the dimensions of the cells.*

FIG. 17. *The tubercle as it appears from in front.*

FIG. 18. *Top of a mature tubercle trichome, showing pitting in the expanded portion of the basal cell.*

FIG. 19. *The same trap, living, just after tripping, with an inclosed bubble of air (left) and two hours later, in the set condition. The collapsed sides (as in Fig. 26) have forced the air bubble to occupy the peripheral space.*

FIG. 20. *Young tubercle trichome with expanding cuticle, the capital cell not yet in its definitive form.*

FIGS. 21–24. *The threshold.*

FIG. 21. *Median region, looking down on the surface of the threshold. The various regions can be recognized in Fig. 22.*

FIG. 22. *Sagittal section of threshold. The regions: velum, middle zone (narrow and dished), the outer zone and the trichomatous zone can be equated with the corresponding zones in Fig. 21.*

FIG. 23. *Threshold, section through 3–4, Text-fig. 5. Reversed with respect to Fig. 22.*

FIG. 24. *View looking into the entrance over the lower portion of the threshold, showing the diaphanous velum.*

FIG. 25. *Surface view of a portion of the middle piece of the door to show the middle line of small cells, with the laterally spreading curves of cells of both courses superimposed.*

FIG. 26. *A set trap and the same immediately after tripping, living. Note that on account of the exhaustion of water, the sides of the trap (left) are mutually quite compressed.*

FIG. 27. *A young, still unopened trap showing particularly the door at this stage of development. The two kinds of trichomes are here well seen.*

FIG. 28. *The same stage as that in Fig. 27, but showing better the newly developed velum. The cuticles of the inner zone of the threshold are not yet fully expanded.*

PLATE I

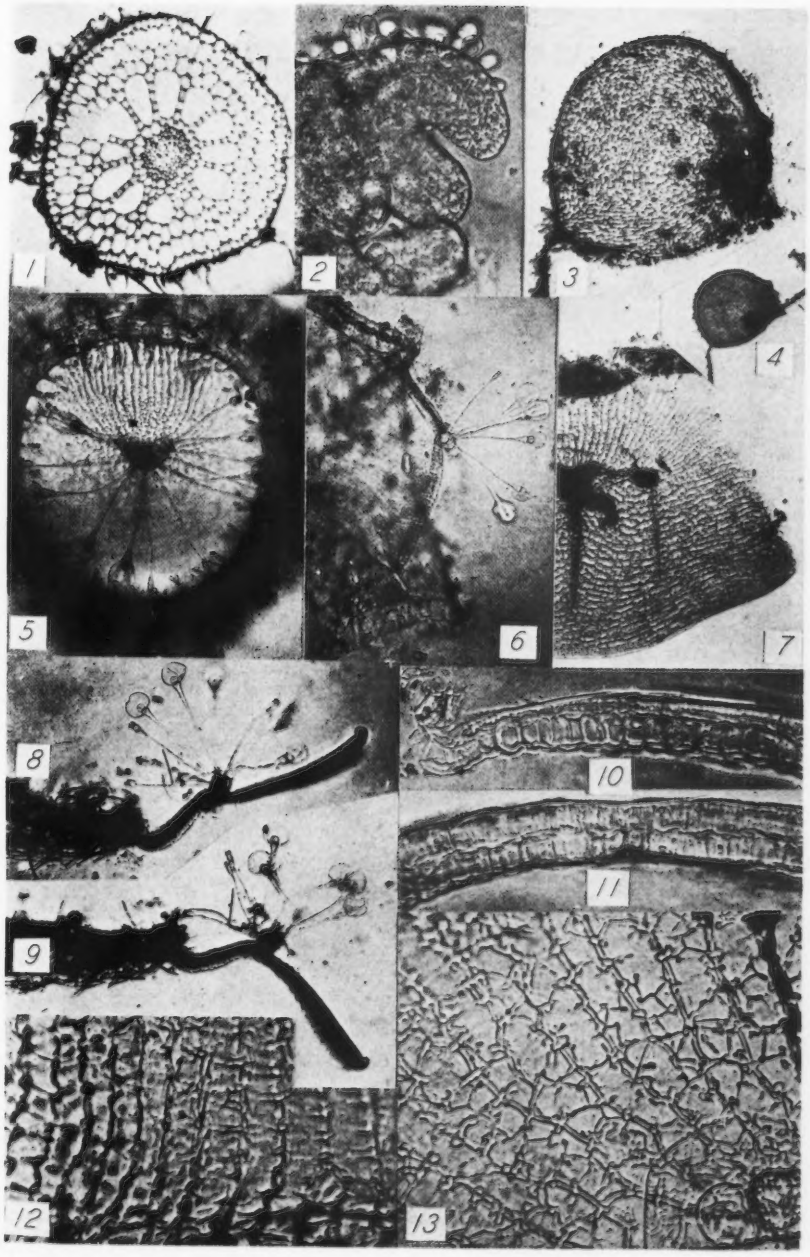
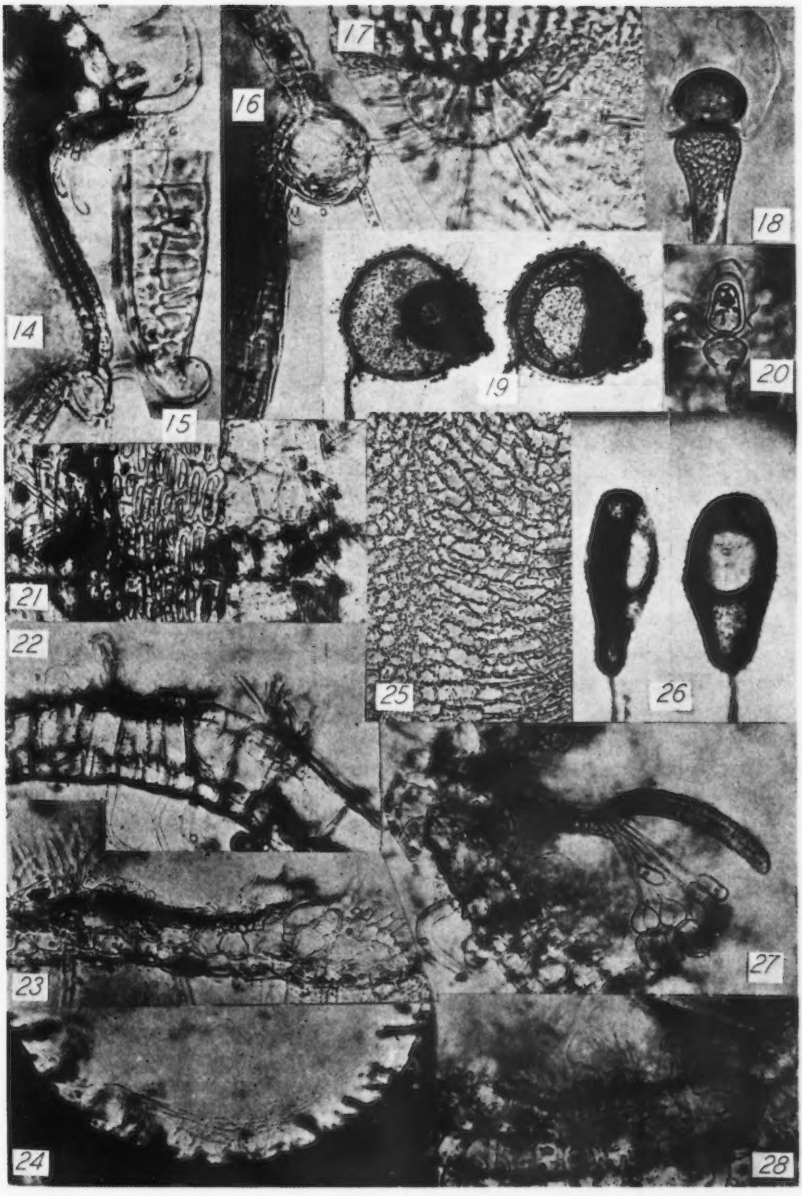


PLATE II



FUNGI ISOLATED FROM MANITOBA SOIL BY THE PLATE METHOD[1]

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Abstract

The fungus-flora of 75 samples of surface soils from various parts of Manitoba has been analyzed. The population of fungi in these soils is abundant and varied despite climatic conditions which might appear unfavorable. Forty-four genera and 100 species of fungi have been identified, and the relative frequency of each determined. It has been found that the methods of soil microbiology give data regarding the parasitic as well as the saprophytic fungi in soils; that *Trichoderma lignorum* parasitizes certain other fungi, and thus may play a part in keeping in check the pathogenic fungi within a soil; and that the addition of chemical fertilizer to a soil results in a prompt and consistent increase in the fungus-flora.

Introduction

The activities of soil micro-organisms are unquestionably of importance to agriculture. Many bacteria and fungi are indispensable in the soil because they decompose organic matter to simple compounds that can be utilized by higher plants. Some are injurious because they bring about unfavorable chemical changes, or because they attack the roots of crop plants.

Bacteria, Actinomycetes, and Fungi* exist in the soil in enormous numbers; they are isolated commonly from Manitoba soils at the rate of 10,000,000 or more per gram of soil. Although no more than 1 to 3% of this total represents fungi, these are of such importance in the soil as to deserve especial attention.

Much work is necessary to establish the identity and relative frequence and importance of the soil fungi in various parts of the world. Manitoba is well situated for an investigation of this problem since the soils are of many and varied types; little work has been done upon soil fungi within several hundred miles; and the wide temperature and moisture changes in the soil make the results of interest. This publication presents a summary of the work undertaken to determine the identity, prevalence, and significance of fungi found in surface soils in Manitoba.

The isolation technique outlined by Waksman (25) and Brierley, Jewson and Brierley (3) has been followed. Each analysis was made on a composite of ten samples taken with a garden spatula to a depth of about four inches after the loose surface layer had been removed. The composite was mixed thoroughly in the field on a sterile canvas, transferred to a sterile paper bag and transported to the laboratory. A moisture test was run at once, and the

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**Fungi as used throughout this paper refers to the Eumycetes or true fungi belonging to the classes Phycomycetes, Ascomycetes, Basidiomycetes, and Fungi Imperfecti.*

result expressed as percentage of the moisture-free soil. The sample, weighing about five pounds, was spread between sterile papers and air dried at about 28° C. for three days, after which it was pulverized, sifted and mixed thoroughly under aseptic conditions. Air-dry moisture was determined and the count recorded on a moisture-free basis. Twenty-five grams of the moisture-free soil was shaken in 250 ml. of sterile water in a litre jar on a mechanical rocker shaker for 30 min. One ml. of a 1 : 5000 dilution was placed in each of eight plates. Four of these were poured with Waksman's peptone-glucose acid agar (8) and four with Czapek's sodium nitrate-sucrose agar (8), both acidified just prior to pouring by the addition of 1 ml. and ½ ml. respectively of 12.5% lactic acid per 100 ml. of medium. Incubation was at 25° C. for from three to five days depending upon the presence or absence of spreaders. Each count represents the average of the results on the eight plates, except in a few cases where spreading types made counting impossible on all plates, when the results on the remaining plates were averaged.

After counting, one plate showing good distribution of colonies, or in some cases one plate from each medium, was selected and each colony picked and transferred to a Czapek's (non-acidified) agar slant or in the case of *Mucor* types, which do not develop well on this medium, to Waksman's agar for study. The other plates were kept and any different-appearing or tardily developing colonies isolated at a later date and marked "extra". In addition three plates of a 1 : 100,000 dilution in sodium albuminate agar (8) and three of the 1 : 50,000 dilution in nitrate-sucrose agar (8), for counts of bacteria and Actinomycetes respectively, were examined for fungi after about 14 days' incubation and isolations made of colonies not recognized as common species.

Isolations were made from 75 samples covering the following soils: garden, orchard and sod, alfalfa and cereal fields at the Agricultural College at Winnipeg; a millet field and a summerfallow at Niverville, south of Winnipeg; a forest, a slough and sod and barley fields at Loni, about 60 miles north of Winnipeg; field and forest at Keewatin, just over the eastern boundary of Manitoba; and forest in the Riding mountains in western Manitoba. The most extensive study was that on two wheat plots carried on over a period of seven months starting April 1932. In the list of species presented below reference to the source is made when a fungus predominated in a particular type of soil. Since the significant differences in the fungus-flora of the soils studied are uncertain the isolations from the various soils are not compared in detail.

The surface soils studied had an average count of 125,000 fungi per gram, or 25 colonies on each plate of 1 : 5000 dilution. Thus about 15,000 colonies were examined macroscopically on the 600 plates for fungi, and an additional thousand or more colonies of fungi were examined on the 450 plates for bacteria and Actinomycetes. The list of species below accounts for the 2,565 transfers which were studied in test tube cultures.

Gilman and Abbott's summary (9) has proved exceedingly useful in this study, and also Waksman's works (23, 24, 25) and other publications, some of which are mentioned in the list of references.

Notes on the Groups of Fungi, and Acknowledgments of Assistance

Many soil fungi are difficult to identify. However, careful and continued study, utilizing when necessary various culture media, gives data by means of which most species may be identified from published descriptions. With some fungi it is necessary to have help from mycologists who have given intensive study to certain groups. The writers are glad to acknowledge with thanks the kind assistance of those mentioned below, who have so generously examined many cultures.

Myxobacteriales, Acrasieae, and Myxomycetes are present in soils, but the special technique necessary to demonstrate the presence of these Myxothallophytes has not yet been applied in Manitoba.

The Phycomycetes are fairly common in soil, in this study representing 137 cultures or 5% of the isolations. All the Phycomycetes recognized belong to the Mucorales. Species of *Pythium*, *Saprolegnia*, *Olpidium* and other lower Phycomycetes are known to occur in soil, and some of them attack the roots of living plants. None of these fungi was obtained in this investigation, because the culture media used were not favorable for their development, and because of the greater relative abundance of spores and mycelium of other fungi in the soils examined.

The Mucorales often are troublesome on isolation plates because their rapid growth in an incubator at 25° C. overwhelms young colonies of other fungi. Fortunately, however, most Mucorales produce a thin growth on Czapek's agar, so that the slower growing fungi may be picked with the overgrowth to tubes of Czapek's agar, where they can often be identified, although plating for purification is necessary in some cases.

Drs. A. F. Blakeslee and Sophie Satin have reported upon several species of *Absidia*, *Syncephalastrum*, and *Zygorhynchus*. *Rhizopus* accounted for more than half of the Phycomycetes isolated.

Ascomycetes which produce asci in culture are rarely found in soil isolations. *Chaetomium*, *Sporormia*, and *Fimetaria* occasionally appear; they are probably more common in soils fertilized with barnyard manure. Yeasts and other lower Ascomycetes appear infrequently in acidified plates.

Basidiomycetes appear to be even scarcer than ascus-bearing fungi in soil isolations. One culture of a smut, a few cultures that might be mycelial stages of Hymenomycetes, and the *Rhizoctonia* stage of *Corticium vagum* were obtained. It might be expected that *Sporobolomyces* would occur in surface soils. Since the commoner species of *Sporobolomyces* are pink, six isolations of pink yeasts were tested, but none discharged spores from sterigmata as *Sporobolomyces* does.

Larger Basidiomycetes often grow conspicuously upon soil, particularly in forests. These fungi are, however, seldom recognizable upon isolation plates from soil.

Fungi Imperfecti constituted more than 90% of the isolations. Species of *Penicillium* are by far the commonest fungi in Manitoba soils, as in soils elsewhere. More than half (1404) of the cultures studied were *Penicillia*. Dr. Thom very kindly named the numerous cultures sent him. The writers may have made mistakes in identifying cultures to be the same as "authentic" cultures determined by Thom, but care has been exercised, and practically every culture has been examined microscopically as well as macroscopically, in comparison with named species and with the assistance of Thom's monograph (20).

Aspergilli were isolated infrequently. Dr. Thom has named a representative culture of each species herein reported.

Fusaria appear commonly in soil isolations. Dr. Sherbakoff identified several species of *Fusarium* and *Cylindrocarpon*, and Dr. W. L. Gordon of the Dominion Rust Research Laboratory at Winnipeg undertook the onerous task of comparing the various cultures, and of identifying them in consultation with Dr. Sherbakoff. Dr. J. E. Machacek, also of the Rust Research Laboratory, has examined critically a number of cultures of various fungi.

Mr. E. W. Mason of the Imperial Mycological Institute, Kew, has given much help with the Dematiaceae and Mr. S. P. Wiltshire of the same Institute has examined certain *Alternarias*. Professor H. H. Whetzel of Cornell University has reported upon several difficult fungi.

Professor J. H. Ellis of the University of Manitoba has given many valuable suggestions relating to soils.

The list of species which follows gives the name of the fungus, with a query where there is doubt as to its identity. The numbers following the name refer to the number of times the species occurred. The approximate percentage of all isolations is indicated whenever the fungus was found to the extent of 1% or more. In the cases of some of the less common species, the fungus was isolated partly or entirely from "extra" plates other than the one or two picked for the standard routine determinations; in such cases the number of "extra" isolations is given. Thus *Helminthosporium sativum* appeared three times in routine isolations, and four times on plates not picked, so the record is "7, four of which were extra". The notes given with species refer to certain diagnostic or other features that seem worthy of record.

List of Species

The fungi are arranged alphabetically under the groups indicated. All specific names are decapitalized.

I Phycomycetes

Absidia glauca Hagem (2, *i.e.*, identified twice only.) Both isolations are of the (+) race and produce zygospores with Dr. Blakeslee's minus test race. *A. glauca* is recorded by Gilman and Abbott (9) from soil in Idaho only in North America, and from three European countries.

Absidia orchidis (Vuill.) Hagem (4). The cultures fit the description of *A. orchidis* very well, but the name has not been verified.

Absidia spinosa Lindner (6, four of which were from sod.) A homothallic fungus with short-cylindric spores.

Absidia spp. (3, not definitely determined.)

Mortierella isabellina Oud. var. ***ramifica*** Dixon-Stewart (2, from forest soils.) Cultures produce a thin gray to drab growth, with branched sporangiophores bearing sporangia 15–20 μ in diameter, with small globose spores 2–2½ μ. The fungus fits the description given by Miss Dixon-Stewart (6) for a fungus isolated from bush soils in Australia.

Mucor ?abundans Povah (4) A *Mucor* near *M. hiemalis*, but with sporangiophores more branched, spores 4–6 × 3–4 μ.

Mucor dispersus Hagem (1) Sporangia 20–70 μ, some with only 3–4 spores; sporangium wall marked with short spines as in *Rhizopus elegans*; spores variable in size, from 7 μ to 15 × 12 μ, globose to slightly oval.

Mucor hiemalis Wehmer (11, especially in forest soil.) Sporangiophores mostly unbranched, but occasionally with one or two branches, sporangia 30–90 μ, spores variable, mostly oval, often 8 × 4 μ, frequently smaller.

Mucor racemosus Fres. (9, the commonest species of *Mucor* in wheat fields.) Sporangiophores branched racemosely; spores fairly large, up to about 10 × 8 μ. The sporangia are encrusted.

Mucor ?sylvaticus Hagem (1) Spores of two sizes, 4 × 3 μ or up to 9 × 6 μ, sporangia small, mostly about 30 μ, chlamydospores present, often terminal.

Mucor spp. (3 unidentified.)

Rhizopus elegans Eidam (9, but see next entry.) Less vigorous in growth than *R. nigricans*, but with rhizoids well developed. Easily recognized by the "elegant wimperig stachelig" markings on the sporangia. Spores 5–8 μ, roundish. Perhaps in reality more common in Manitoba soils than is *R. nigricans*. A number of cultures were paired in an effort to obtain zygospores, but none were produced.

Rhizopus nigricans Ehr. (67 cultures, over 2%, credited to this species, but at first it was not distinguished from *R. elegans*.) *Rhizopus* is common in soil and troublesome on isolation plates.

Syncephalastrum racemosum Cohn in Schroet. (12, in two isolations from wheat fields.) Cultures white, then yellow and finally dark brown. Sporangiophores branched, bearing globose heads of sporangia with spores globose, 3–5 μ or oval and up to 8 × 4 μ. Apparently not previously reported from soil.

Zygorhynchus heterogamus Vuill. (1, garden.) Zygospores comparatively large, 50–75 μ, becoming black, with coarse rough markings. Spores from sporangia roundish, up to 4 μ in diameter.

Zygorhynchus* ?*moelleri Auct. Amer., non Vuill. (1, forest soil.) Zygospores 30–55 μ, golden brown with pyramidal markings 4–5 μ high. Spores from sporangia 4–5 × 2–3 μ. Dr. Blakeslee states that this fungus resembles the form which has gone under the name *Z. moelleri* in America, which differs from the species called by this name in Europe.

Zygorhynchus sp. (1, forest soil.) Dr. Blakeslee writes that it is neither *Z. vuilleminii* nor *Z. moelleri*. The transfer kept at Manitoba has been lost. Waksman (23) reports *Z. vuilleminii* to be common in subsoils. Species of *Zygorhynchus* apparently are rare in surface soils in Manitoba.

II Ascomycetes

Chaetomium* ?*funicola Cke. (6, three of which were extra.) These cultures are placed here with some doubt. The perithecia produce setae which are not incrusted, sometimes branched. Asci not clearly seen, but the spores are often in aggregations and seem to have been produced in asci. Spores very abundant, sometimes 5–7 × 3–3½ μ, but often as small as 4 × 2 μ. The cultures produce dark floccose mycelia studded with perithecia.

Chaetomium* ?*spirale Zopf (1) Perithecia bear masses of spiral hairs, somewhat incrusted. Ascospores 11–15 × 6–8 μ, olive green, ellipsoid with narrowed ends.

Fimetaria fimicola (Rob.) Griff. and Seaver (1) Perithecium pyriform, nearly smooth; ascospores 18–22 × 12–13μ, greenish, with a hyaline envelope.

Gymnoascus* ?*reessii Baranetz. (1, wheat field.) Culture purplish-red, producing numerous roundish perithecia 125–200 μ consisting of loose wefts of hyphae with ends projecting, and containing roundish asci 7–10 μ in diameter with 8 small oval spores 3–4 × 2 μ. This fungus is apparently a *Gymnoascus*, but does not fit the species *G. reessii* well.

Saccharomyces (?) spp. (9 cultures from routine isolations on acidified agar.) Some forms are pink, but in no case was *Sporobolomyces* found.

Sporormia fasciculata Jensen (2) Perithecia are produced readily on Czapek's agar. The asci are 40–55 × 20–27 μ, and contain eight four-celled spores which are 28–32 × 6–7 μ, brown. These spores readily separate into single cells 7–10 × 6–7 μ. The colony has a purplish and white appearance, and the perithecia may be aggregated into small clusters. Since the fungus resembles the description of *Sporormiella nigropurpurea* Ell. and Ev., described on dung, cultures were made on sterilized horse dung, upon which the fungus grows well, but the asci are shorter and wider than reported for *Sporormiella*.

Sporormia sp. (2) Cultures resemble the preceding species, having a purplish color. One isolation produced perithecia readily (possibly because the culture was contaminated), the other isolation developed perithecia only when yeast extract was added to the culture medium. Both produce asci longer than in *S. fasciculata*, being 70–110 × 14–20 μ, the spores 20–25 × 6–7½ μ, brown. The perithecia are single, not in clusters.

III Basidiomycetes

Rhizoctonia solani Kuehn (1, wheat field.) This fungus is known to be common in soils, but is not obtained often by the isolation methods used in soil microbiology. Pratt (17) isolated it five times from Idaho soils.

Ustilago sp. (1) Produces a rather slimy cream-colored growth, with areas of thin white mycelium. The spores or cells are mostly 8–24 × 2–3½ μ, and show budding. Dr. W. F. Hanna examined the culture and found it to resemble *U. zeae*, but it could not be determined definitely.

IV Fungi Imperfecti

A. Moniliaceae

Acrostalagmus cinnabarinus Corda (5, three of which were extra isolations.) Colonies cinnabar or brick red, becoming sometimes white mycelioid. The conidiophore verticils are often 4–5 rayed, but sometimes have only two or three branches. The spores are mostly 3–5 × 1½–2 μ, but a few spores can be found 6–10 × 2–3 μ. This fungus seems to fit the species rather than the variety *nanus*.

Aspergillus flavipes (Bain. and Sart.) Thom and Church (25, 1%.) This has proved to be the commonest *Aspergillus*, but was obtained almost exclusively from wheat fields. It produces a yellow growth with amber drops and a "forest" of long conidial heads.

Aspergillus flavus Link group (4, one of which was extra.) The cultures were found to produce kojic acid.

Aspergillus fumigatus Fres. (9, especially from old sod.) This *Aspergillus* occurs on old plant parts, and sometimes causes the death of young chickens. Cultures grow rapidly at 38° C.

Aspergillus niger van Tiegh. (3, one of which was extra.) This fungus is more common in soil in southern parts of North America. Werkenthin (27) found it to be the most frequent soil fungus in Texas; Le Clerg (14) reports *A. niger* to constitute more than 1% of the fungus-flora in Colorado soil; Miss Todd (22) has recently found it to represent about 40% of the surface soil flora in Oklahoma. *Aspergillus niger* is conspicuous by its rarity in surface soils in Manitoba. It is not easily mistaken or overlooked. It provides an example of a soil fungus with apparent variation in regional distribution.

Aspergillus okazakii Okazaki (2, wheat field.) A member of the *A. candidus* group.

Aspergillus ustus (Bainier) Thom and Church (6, one of which was extra.) The cultures are dark gray; considered by Dr. Thom to be perhaps between *A. ustus* and *A. insuetus*.

Aspergillus versicolor (Vuill.) Tirab. (1, wheat field.) Reverse of colony red to purplish.

Botrytis cinerea Pers. (3, one of which was an extra isolation.) The three cultures show the usual variation in spore and sclerotium formation.

Botrytis terrestris Jensen (3, one of which was extra.) Professor Whetzel agrees that this fungus is similar to, or identical with, the *B. terrestris* of Jensen (13), although he does not consider it to be a good *Botrytis*. The fungus produces a gray-brown restricted growth, with a more or less velvety surface and a few dark drops, cultures reddish-chestnut-brown below. Spores 3–4 × 2½–3 μ, forming masses at the ends of the conidiophores.

Cephalosporium acremonium Cda. (19, nearly 1%.) The *Cephalosporium* which produces a pink to salmon, slimy growth on potato dextrose or malt agars, with narrow cylindric spores 3–6 × 1–2 μ, is interpreted to be *C. acremonium*.

Cephalosporium ?curtipes Sacc. (13) A white fungus with spores in heads, 4–9 × 2–4 μ. Spores rather small for *C. curtipes* as described.

Cephalosporium humicola Oud. (4) Apparently this species; conidiophores rather long, spores roundish or oval, 3–4 × 2–3 μ. The colonies are rather paler in color than in *C. acremonium*.

Cephalosporium sp. (11) Several times a *Cephalosporium* has been isolated which produces spores 6–12 × 2–3 μ, grows well on Czapek's agar producing a wrinkled damp colony sometimes salmon, sometimes yellow, sometimes showing both colors. This fungus may be the same as reported by Waksman (23, p. 133) as "G.23".

Cylindrocarpon ?candidum (Link) Wollenw. (1, extra.) Culture white, producing cream-colored masses of conidia, 42–58 × 5–6½ μ, mostly three-septate.

Cylindrocarpon candidum var. ***majus*** Wollenw., probably (6, from wheat field.) Cultures somewhat darker in the variety. Spores 50–64 × 5–6 μ, mostly three-septate, produced in cream-colored masses.

Cylindrocarpon didymum (Hart.) Wollenw. (11, especially in wheat field.) Cultures brown to yellow, sometimes pinkish, and usually with an odor resembling that of a broken young stem of maize. Spores produced in buttery yellow masses, one-septate, 20–28 × 3½–4½ μ.

Cylindrocarpon ?heteronemum (B. and Br.) Wollenw. (2, wheat field.) Cultures white to brownish, spores 16–20 × 3–4 μ, commonly with one septum.

Cylindrocarpon ?macrosporum (Fres.) Wollenw. (4, wheat field.) Cultures brownish, chestnut brown on reverse; spores 20–40 × 5–7 μ.

Dactylium dendroides (Bull.) ex Fr. (1, forest soil.) Produces a *Mucor*-like growth with verticillate conidiophores bearing three-septate spores 20–30 × 9–12 μ. It is curious to note that the fungus on Czapek's agar produces the purple-red color characteristic of *Hypomyces rosellus*; on potato dextrose agar the culture is only very faintly pink. Not a true soil organism, but found in Manitoba upon old *Hymenomycetes*.

Fusarium coeruleum (Lib.) Sacc. (1, wheat field.) This fungus is a common cause of dry rot of potatoes in Manitoba.

Fusarium culmorum (W. G. Smith) Sacc. (16, all from cereal fields, and 9 of these from a plot which had been inoculated with the organism.) This fungus, parasitic to cereals, can be isolated by the methods of soil microbiology. An isolation was found to be parasitic to wheat, as recorded below.

Fusarium culmorum var. ***cereale*** (Cke.) Wollenw. (3 isolations from soil previously inoculated with cultures of *F. culmorum*.)

Fusarium equiseti (Cda.) Sacc. (2)

Fusarium herbarum (Cda.) Fr. var. ***avenaceum*** (Fr.) Wollenw. (1, garden.)

Fusarium moniliforme Sheldon (3 identified.) This *Fusarium* and its varieties are probably fairly common in soils. Found also by Henry (10) in soil from Brandon, Man.

Fusarium oxysporum Schlecht. (10, from garden and old sod, rarely from wheat fields.) Found frequently in Manitoba as a parasite in wilted potatoes. *Fusaria* belonging to the *elegans* section are common in soil, but several have not been identified.

Fusarium poae (Peck) Wollenw. (1).

Fusarium reticulatum Mont. (2)

Fusarium sambucinum Fckl. (2, wheat field.)

Fusarium scirpi Lamb. and Faut. (9, various soils.)

Fusarium scirpi var. ***acuminatum*** (Ell. and Ev.) Wollenw. (21, 1%.) Common in various soils. The cultures are floccose and bright red.

Fusarium solani (Mart.) var. ***martii*** (App. and Wollenw.) Wollenw. (9, found especially in wheat fields.) The cultures develop the typical green to blue color.

Fusarium sporotrichioides Sherb. (2, from soils with cereals.)

Fusarium vasinfectum Atkinson (24, 1%, especially from sod and alfalfa field, but also in garden, orchard and grain field.)

Fusarium vasinfectum var. ***lutulatum*** (Sherb.) Wollenw. (21, 1%, especially in garden.) Aerial mycelium is often replaced by a yellowish-pink mass of pseudopionnotes.

Fusarium vasinfectum var. ***zonatum*** (Sherb.) Wollenw. (1, wheat field.)

Geotrichum candidum Link (2) · Cultures on agar are white or pale cream color, with a broadly spreading, rather velvety but thin mycelial growth, and with the ends of the hyphae breaking up into segments (spores) 5–12 × 3–4 μ, cylindrical, ends obtuse. These cultures were carried as "*Monilia* sp." until tested on sterilized leaves, where small white tufts were produced, and the fungus seems to agree with *Geotrichum candidum* as recorded by Lindau (15) and by Sumstine (19).

Gliocladium atrum Gilman and Abbott (2) This fungus produces round *Gliocladium* heads with spores about 3 × 2 μ on penicillate conidiophores, but, as Gilman and Abbott state, the mycelium is so dark as to place it in the *Dematiaceae*.

Gliocladium catenulum Gilman and Abbott (1, an extra from sod.) Colonies white then green, with long heads of spores, reverse yellowish. Spores 5–8 × 2½–3 μ.

Gliocladium penicilloides Corda (8) An attempt was made to separate in this species the paler, more cream-colored cultures with spores somewhat smaller, but unquestionably this "species" merges into *G. roseum*.

Gliocladium roseum (Link) Bainier (42, nearly 2%.) Common in various soils. The cultures are white then salmon pink. Spores in heads, 4–7 × 2½–4 μ.

Hymenula affinis (Fautrey and Lambotte) Wollenw. (13, from garden and wheat field.) This fungus produces a pale salmon, slimy layer, with abundant spores with one or two cells.

Metarrhizium sp. (28, 1%; all cultures except one from wheat fields.) This fungus is easily recognized by the production on Czapek's agar of a floccose mass of pure white mycelium, which later produces very dark areas of spores. The reverse is pale yellow. Spores 5–8 × 2½–3 μ. Some species of *Metarrhizium* are parasitic upon insects. A specific name for this fungus has not been found.

Monilia ?geophila Oud. (1) This *Monilia*, with a bright yellow growth and conidiophores breaking up into spores, resembles *M. geophila* except that the spores are larger, being 6–10 × 4–6 μ on Czapek's agar. On gelatin the spores are variable, and the gelatin is liquefied.

Monilia sitophila (Mont.) Sacc. (2) This fungus grows so rapidly and conspicuously that it cannot be overlooked in cultures. Evidently it is not abundant in soil in Manitoba.

Penicillium albidum Sopp (1, from sod.) Determined by Thom as possibly this species. The culture is white at first, then green to olive green, zonate and radiately wrinkled, reverse orange to buff. Conidiophores enlarge considerably at apices.

Penicillium aurantio-brunneum Dierckx (3, millet field.) The cultures produce a brilliant amber color in the medium.

Penicillium braziliense Thom (9, one of which was extra.) This *Penicillium* is easily recognized by its chalky-white appearance, with usually a faint tinge of pink. The reverse of the culture is yellowish; one culture which remained for some months in the refrigerator developed a dark green color in spots on the reverse, and this color persisted in transfers.

Penicillium canescens Sopp (3, wheat field and forest soil.) This fungus, identified as "close to *P. canescens*", has been found previously in soil in Norway and England.

Penicillium carmino-violaceum Dierckx (27, 1%.) A beautiful species which colors the medium shades of red to violet.

Penicillium chrysogenum Thom (152, 6%.) *Penicillia* belonging to the *chrysogenum* series are common in soil. Drops are produced on the surface which are commonly yellow, sometimes nearly colorless. The reverse is yellow to brown. Milk is digested with production of a bright yellow color.

Penicillium ?duclauxi Delacr. (1, wheat field.) The fungus scarcely grows upon Czapek's agar, but produces a coremiform growth on potato dextrose agar.

Penicillium funiculosum Thom (11) Produces reddish coremia with masses of green spores; medium colored deep red.

Penicillium frequentans Westl. (11) Not found to be common in Manitoba soils. The cultures resemble those of *P. rugulosum* superficially, but lack the bright yellow margin in reverse.

Penicillium griseo-roseum Dierckx. Certain cultures agree with the description of this species, but are counted with the related *P. chrysogenum*.

Penicillium intricatum Thom (299, 12%.) This is one of the commonest organisms in the various soils examined. It produces a funiculose mass of hyphae at first white or gray to brownish and without spores, becoming smoky green from spores; reverse brownish.

Penicillium janthinellum Biourge (217, 9%.) Also very common in Manitoba soils. Thom (20) calls the *P. janthinellum* series "the 'soil' *Penicillia*".

Penicillium kapuscinskii Zaleski (1) Our culture resembles *P. intricatum*, and possibly we may have referred a few cultures erroneously to the latter species.

Penicillium lilacinum Thom (21, nearly 1%.) This species sometimes is striking, with a lilac surface and yellow reverse, but various shades of colors are produced by different isolations. Three cultures show the *Isaria*-like hyphal columns mentioned by Thom (20, p. 335) as occurring with the form *Spicaria violacea*.

Penicillium luteum Zukal, group (3, garden.)

Penicillium purpurogenum Stoll (4) The surface is yellow-granular at first, and the reverse is purplish to red.

Penicillium purpurrescens Sopp (3, wheat field.) Dr. Thom writes that this fungus agrees with Sopp's description. Surface green, powdery in older cultures; reverse purplish to very dark; spores echinulate, $3\frac{1}{2}$–$4\frac{1}{2}$ μ.

Penicillium restrictum Gilman and Abbott (45, 2%.) This fungus, with restricted growth and dark surface, is common. Usually the reverse is colorless, but in ten of our cultures the reverse is brownish.

A form that seems nearest *P. restrictum* is commoner: we have isolated it 95 times (nearly 4%), especially from forest soils. The spores are globose and rough; the colony is dark colored, often somewhat green. It differs markedly in appearance from the typical form in the thin growth that is rather powdery from spores and sometimes zonate.

Penicillium rugulosum Thom (55, 2%.) Spores oval, slightly rough; colonies brilliant yellow at sides of slants in test tubes, and in reverse. Thom (20) reports *P. rugulosum* as a common parasite of *Aspergilli*. Tests were made by growing *P. rugulosum* with colonies of *Fusarium culmorum*, *Helminthosporium sativum* and *Rhizopus*, but in no case was parasitism by this strain of *P. rugulosum* evident.

Penicillium rugulosum var. ***atricolum*** (Bain.) Thom. Two or three cultures produce the restricted growth and paler reverse of this variety.

Penicillium simplicissimum (Oud.) Thom (26, 1%, especially from forest soil.) This species resembles one strain of *P. terrestre* superficially, but the conidiophores are different.

Penicillium spinulosum Thom (2) This monoverticillate *Penicillium* has rough spores and a dull green, loose conidial area.

Penicillium terrestre Jensen (307, 12%.) This has been found to be the commonest *Penicillium* in soils cropped with wheat. One strain has a floccose surface, another is powdery; young cultures often have an aromatic odor resembling that of bananas. All cultures become gray when older. The reverse, on Czapek's agar, varies from colorless to buff, pink, or brownish.

Penicillium thomii Maire (66, nearly 3%, especially common in forest soil.) This variable fungus is characterized by the production of dense masses of sclerotia which may be salmon, pink, yellowish or brownish. The reverse is nearly colorless to brown. In some strains abundant pale greenish conidial areas are produced sooner or later, in others no noticeable conidial production occurs. The conidia are oval, on monoverticillate conidiophores. Cultures on Czapek-Dox solution with 3% glucose produce in some cases a yellow color in the solution near the top; other strains produce no color.

Penicillium variabile Sopp (4) One of the *P. purpurogenum* series.

Penicillium viridicatum Westling, series (6, wheat field.) Cultures somewhat zonate above and yellow to reddish in reverse.

Penicillium spp. (about 30, 1%.) A few mixed cultures with an overgrowth of *Rhizopus*, *Trichoderma*, etc., were discarded. They were probably for the most part common *Penicillia*.

Scopulariopsis brevicaulis (Sacc.) Bainier (5, four of which were extra.) This fungus is known to be common in Manitoba upon old hay and spoiled silage, but it was not found often in cultures from the soil, partly because it starts somewhat more slowly than many other soil fungi. The spores are rough and comparatively large.

Scopulariopsis rufulus Bainier. (1) Culture coffee-brown, darker below; spores 5–7 × 5 μ, faintly tuberculate.

Sporotrichum roseum Link (9, six of which were extra isolations.) As Miss Dale (5, p. 53) states, this fungus is a variable and slowly growing form; it is apt to be swamped by other fungi before it develops noticeably. Colonies white to reddish or purplish, dark purplish brown below. Spores small, 3–4 × 2–3 μ.

Trichoderma album Preuss (21, nearly 1%.) This white *Trichoderma* commonly makes very little growth on Czapek's agar, but some strains placed with this species grow upon it readily. Although the fungus remains white or whitish upon the agar media tested, abundant green or greenish masses of spores are formed by some strains upon sterilized leaves or sticks of *Acer negundo*.

Trichoderma glaucum Abbott (4) Occasionally one finds a *Trichoderma* which resembles *T. album* in appearance, but becomes yellowish to pale greenish, and has larger spores (about 4 × 3 μ) than *T. album*.

Trichoderma koningi Oud. (70, 3%.) A somewhat broad interpretation of this species has been used to include the more or less floccose green *Trichodermas* with oval spores.

Trichoderma lignorum (Tode) Harz (22, 1%.) This fungus is fairly common in soils. It produces a thin growth of mycelium on Czapek's agar, with tufts which become green and bear many small spores 2½–4 μ, roundish or slightly oval. The parasitism of this fungus on other fungi is discussed below.

Verticillium ?glaucum Bonord. (6, two of which were extra isolations.) The colony characters are as described by Waksman (23, p. 136), being yellow-green above, olive green below. Spores are produced very abundantly, and are 3–7 × 2–3 μ. Conidiophores branched singly or in whorls, producing heads of spores. *V. glaucum* is described as having globular spores; this fungus may be nearer to *V. alboatrum* Reinke and Berth.

Verticillium ?terrestre (Link) Sacc. (6). A pure white floccose growth is produced, with more or less verticillately branched conidiophores bearing heads of small spores 3–5 × 2–3 μ.

B. Dematiaceae

Alternaria tenuis Nees, group (7) Spores about 28–45 × 10–12 μ.

Alternaria spp. (31, more than 1%.) Various forms of *Alternaria* appear commonly in cultures from soil.

Botryotrichum atrogriseum van Beyma (2) Cultures dark gray on surface, nearly black below. The original cultures showed spores from 13–22 μ in diameter, but in later transfers they are only 11–16 μ.

Botryotrichum piluliferum Sacc. and March. (2, garden.) Spores 9–14 μ, globose, smooth, colony dark gray above, yellowish-brown below.

Cladosporium herbarum (Pers.) Link (69, nearly 3%.) Forms resembling *C. epiphyllum* and *Hormodendron cladosporioides* were found. The spores are sometimes smooth, sometimes rough; commonly one-celled, but often with two or three cells. A common and variable fungus in the soil. Fungi belonging to other species of *Hormodendron* are sometimes found, but were not identified.

Coniosporium arundinis (Corda) Sacc. (1) The spores are 5–8 μ wide in one diameter, about 4 μ in side view, marked with a hyaline line.

Helminthosporium sativum Pam., King, and Bakke (7, four of which were extra, all from wheat fields.) Identifications made or verified by Dr. J. E. Machacek. There is some variation in the appearance of the different cultures, but the spores are similar in all cases, 30–80 $\times$ 17–27 μ, the larger spores mostly five to seven-celled, olive green to black. The parasitism of this fungus was demonstrated as described below.

Mycogone nigra Jensen, probably not the same as *Monotospora nigra* Morgan. (18, principally from wheat field.) This fungus which Jensen (13) obtained from soil, is probably the same as the fungus referred doubtfully to *Basisporum gallarum* by Miss Dale (4, 5) and Waksman (23). The cultures produce a typical growth at first yellow then nearly black, the reverse becoming very dark and zonate. The spores have a small pale basal cell.

Stemphylium spp. (5)

Torula convoluta Harz (3, garden.) A dark fungus which produces spores 4–6 $\times$ 4–5 μ, and these collect in small heads.

Trichocladium asperum Harz (2, garden.) Spores two-celled, the upper cell larger.

C. Other Fungi Imperfecti

Colletotrichum sp.? (4) Cultures first pinkish, with an abundant, thin, moist layer of spores, then becoming black with sclerotium-like masses of mycelia, which sometimes bear small setae. Spores 10–12½ $\times$ 3–4 μ, sometimes smaller, often one-septate. The fungus somewhat resembles *C. atramentarium*, but is probably not that species.

Coniothyrium spp. (14) Three or more species of Coniothyrium have been isolated. The dark spores in some cases are small, 3–5 $\times$ 2 μ, or roundish, 3½–4 μ; in one isolation the spores are 5–8 $\times$ 3–4 μ. These fungi no doubt represent stages of *Pyrenomycetes* which grow on old vascular plants or their parts. It would be difficult or impossible to apply specific names with any meaning to fungi such as *Phoma* or *Coniothyrium* without knowing the hosts.

Cytospora sp.? (1, from river bank.) Stromatic masses of pycnidia with allantoid, pale olive colored spores 3–5 $\times$ 1 μ. This fungus perhaps developed in nature upon some twig.

Pestalozzia sp. (1, forest soil.) A fungus belonging to the section *quadriloculare*, spores 18–22 × 6–7 μ, with three setae on the end about as long as the spore; two centre cells brown, end cells hyaline. Probably entered the soil from some part of a vascular plant.

Phoma spp. (29, 1%.) The *Phoma* stages of *Pyrenomycetes* which probably grow in nature upon higher plants or their remains are fairly frequently isolated from soil. Pycnidia are often produced abundantly in cultures, and spores are found in some cases as small as 2–4 × 1½ μ, in others as large as 14–19 × 2½–3½ μ. Some of these may represent species of *Phyllosticta*.

Undetermined fungi (about 300, 12%.) In making large numbers of isolations from soil, one inevitably fails to determine every fungus. About 130 cultures of fungi (*Fusaria*, *Trichodermas*, etc.) were mixed, lost before identification, etc.; these were probably for the most part species already included on previous pages. There still remain, however, about 170 cultures, belonging to perhaps a score or more of species, for which names could not be obtained. Some of these fungi have not been induced to form spores; others produce only microconidia; others belong to very difficult groups. Some of these fungi perhaps represent haploid phases of *Hymenomycetes* or *Pyrenomycetes*, which will not develop perfect stages without mating with the proper haploid of another sex, and there are few clues.

Cultures have been kept of most of the species, known and unknown, and further work may illuminate some of them more clearly.

SUMMARY OF FUNGI ISOLATED

Genus	Number of entries	Number of isolations	Genus	Number of entries	Number of isolations
Absidia	4	15	*Monilia*	2	3
Mortierella	1	2	*Penicillium*	25	1,404
Mucor	6	29	*Scopulariopsis*	2	6
Rhizopus	2	76	*Sporotrichum*	1	9
Syncephalastrum	1	12	*Trichoderma*	4	117
Zygorhynchus	3	3	*Verticillium*	2	12
Chaetomium	2	7	*Alternaria*	2	38
Fimetaria	1	1	*Botryotrichum*	2	4
Gymnoascus	1	1	*Cladosporium*	1	69
Saccharomyces	1	9	*Coniosporium*	1	1
Sporormia	2	4	*Helminthosporium*	1	7
Rhizoctonia	1	1	*Mycogone*	1	18
Ustilago	1	1	*Stemphylium*	1	5
Acrostalagmus	1	5	*Torula*	1	3
Aspergillus	7	50	*Trichocladium*	1	2
Botrytis	2	6	*Colletotrichum*	1	4
Cephalosporium	4	47	*Coniothyrium*	1	14
Cylindrocarpon	4	24	*Cytospora*	1	1
Dactylium	1	1	*Pestalozzia*	1	1
Fusarium	17	128	*Phoma*	1	29
Geotrichum	1	2	*Unknown*	—	300
Gliocladium	4	53	Totals:		
Hymenula	1	13	44 genera known	121 entries	2,565 cultures
Metarrhizium	1	28			

It may be noted that in ten cases the genus was determined, but the one or more species found were undetermined. There are also a few varieties which are not counted in the entries for species. The number of species actually named is 100 (exclusive of varieties) and a few of these specific names are questionable. The total number of distinct species of fungi actually found in the soil is in the neighborhood of 150.

The Numbers of Fungi in Surface Soils of Manitoba

The Manitoba soils supporting plant life gave counts of fungi varying from 18,000 to 350,000 per gram. A sample taken in winter from below the average summer water line on the bank of the Red river showed only 133 and two others from above that line gave 1,700 and 3,000 respectively. The highest counts were obtained from forest soils, although an alfalfa field sampled in January gave 246,000 and in June 195,000 molds. Details of the relative numbers and kinds of fungi in the various types of soil investigated in a preliminary way are not presented since the significant differences are not clear.

The most extensive study thus far made was conducted with soil from two adjacent wheat plots. From 1919 to 1931 these two plots were not distinct, but constituted a plot run on a three-year rotation of wheat, wheat, fallow, without fertilizer or barnyard manure. In 1932 the plot was divided, half being left unfertilized, and half fertilized at seeding with ammonium phosphate containing 10% nitrogen and 48% phosphorus pentoxide, at the rate of 45 lb. per acre in one application. Twenty samples were taken from each of the two plots which differed, so far as known, only in the addition of fertilizer to one of them during the course of the tests. Table I presents the counts, and indicates clearly that the addition of fertilizer gives an immediate and consistent increase in population of fungi.

The irregular fluctuations in numbers of fungi in a soil are not easily explained. Soil temperature and moisture no doubt play a part; it would seem that the number of fungi rises soon after moisture increases, and *vice versa;* with higher soil temperatures, the fungi may increase. It will be noted that the numbers of fungi fluctuate in a similar way in both the fertilized and unfertilized plot, the former numbers remaining higher.

Newton (16), Erdman (7), Brierley (2, 3) and others have discussed these seasonal fluctuations in numbers of fungi in soil. Plate counts at least give evidence of the sensitiveness of the soil population to environmental changes.

Although there is definite fluctuation in numbers of fungi in soil, little evidence was found that the cold of a long Manitoba winter reduces the number of viable spores in soil. Thus a garden plot gave counts of 74,000 fungi per gram in December, and 50,000 in late June; an orchard soil 91,000 in January and 87,000 in June; and alfalfa field 246,000 in January and 195,000 in June. Erdman (7) found a maximum count of molds in an Iowa soil in January.

The numbers of fungi in Manitoba soils are similar to those found in other soils in North America; indeed they are higher than many recorded by Waksman (25) but not as high as Miss Todd (22) reports for Oklahoma.

TABLE I

NUMBERS OF FUNGI DURING SEASON IN PLOTS FERTILIZED* AND UNFERTILIZED

Date sample taken 1932	Moisture, % (dry basis)	Temperature,** ° F.	Numbers of fungi per gram of soil	
			Unfertilized	Fertilized*
April 22	42.9		46,000	44,000
April 29	43.7	36.0	51,000	39,000
May 6†	37.0	41.9	78,000	86,000
May 13	35.5	52.9	80,000	138,000
May 20	34.6	54.6	62,000	85,000
May 27	30.1	54.1	61,000	70,000
June 2	29.0	57.1	37,000	42,000
June 10	23.3	67.4	91,000	190,000
June 20	37.6	67.7	107,000	288,000
June 25	30.9	65.3	102,000	127,000
July 4	29.8	63.1	152,000	212,000
July 14	27.0	65.4	65,000	133,000
July 25	22.1	71.9	98,000	133,000
Aug. 4††	17.7	68.2	70,000	129,000
Aug. 16	13.9	68.9	25,000	58,000
Aug. 31	39.1	71.2	18,000	47,000
Sept. 13	22.2	—	127,000	96,000
Sept. 29	34.4	50.5	68,000	100,000
Oct. 13	34.4	43.6	48,000	78,000
Nov. 11	43.4	32.0§	87,000	60,000
Average of 18 tests after fertilizer applied			76,400	115,100

**Ammonium phosphate drilled in with seed.*

***Average of readings taken twice daily at 8 a.m. and 4 p.m. covering the period since previous sampling. The thermometer was in a perforated wooden case placed in the ground so that the mercury bulb extended 3 in. below the surface.*

†Plot seeded and fertilized May 2. First sample after fertilizing.

††Crop harvested August 4.

§Temperature at final sampling.

Parasitic Fungi Isolated from Soil

Plant pathologists have found that a considerable number of parasitic fungi remain alive in soils, sometimes for years. Fungi causing root rots of cereals, flax, and other cultivated plants are particularly troublesome and persistent in soils. Species of *Fusarium* are important plant pathogens capable of saprophytic existence in soil, and *Fusaria* are obtained frequently from isolations made by soil microbiologists. LeClerg (14) reports that 23% of the fungi isolated from Colorado soils were *Fusaria;* Gilman and Abbott (9) list 27 species of *Fusarium* as having been isolated from soil. These fungi are common also in Manitoba soils, and *F. culmorum*, known to cause root rots, is one of the commonest. A culture of *F. culmorum* isolated from a wheat field was found to be parasitic to wheat, as described in the next section.

The methods of soil microbiology have been subject to some criticism because they have failed to demonstrate the occurrence in soil of *Helminthosporium sativum*. This fungus appeared, however, several times in isolations from wheat fields, and was found to be parasitic to wheat.

Rhizoctonia solani, another fungus parasitic to many plants, was isolated once from Manitoba soil. This fungus does not produce spores in its ordinary vegetative growth, and the *Corticium* stage seldom is found in Manitoba, so that *Rhizoctonia* is not likely to be isolated as frequently as its prevalence in soil might warrant.

Fungi parasitic to aerial parts of plants sometimes may find their way into a soil sample: thus the *Cytospora* and some of the isolations of *Phoma* and *Coniothyrium* perhaps represent the imperfect stages of fungi more or less parasitic to plants. *Botrytis cinerea* also may develop parasitically upon higher plants.

The methods of soil microbiology yield data of value regarding the parasitic fungi in a soil, and these data have some quantitative value. For example, about 7000 colonies developed from 40 isolations from the wheat plots; seven of these were found to be *Helminthosporium sativum*. We may conclude that *H. sativum* exists in this soil in a proportion of the order of 7 : 7000 total. The average number of fungi found in the wheat field was 90,000 per gram of surface soil: the calculation indicates that each gram of a soil cropped to wheat for some time in Manitoba may contain 90 viable spores or bits of mycelium of *H. sativum*. Henry (12) has studied this fungus in Alberta soils.

Soil Fungi Parasitic upon other Fungi

When one considers the possible prevalence in the soil of fungi parasitic to crop plants, it becomes evident that nature must strike a balance in some way, or crops could not be grown. Sanford and Broadfoot (18) and Henry (11) have discussed this problem as pertaining to soils in western Canada.

During July, 1932, the writers made isolations from a soil which was used as a "disease garden" to test resistance of barley varieties to soil fungi. Plots had been inoculated heavily with pathogenic *Fusaria* and *Helminthosporium*, and adjacent plots left as checks. The barley showed no obvious difference on the two plots. Soil isolations were made, and colonies of *Fusarium culmorum* started in considerable numbers upon plates from the inoculated soil, but it was striking to note the rapidity with which *Trichoderma lignorum* overwhelmed and destroyed these colonies of *Fusaria* on petri dishes. *Trichoderma* previously had been considered as merely a rapidly spreading mould; it now appeared as a beneficial parasite. Before tests were made to corroborate the observation, Weindling (26) published a paper which shows clearly that *T. lignorum* parasitizes certain soil fungi (*Rhizoctonia solani*, *Phytophthora parasitica*, *Pythium* spp., *Rhizopus* spp., and *Sclerotium rolfsii*).

A test of the efficacy of *T. lignorum* in preventing injury by pathogenic fungi to wheat in soil was undertaken in the following manner. Pots of soil

were sterilized at 15 lb. pressure for three hours, and to them were added (1) heavy inoculation of *Fusarium culmorum*, soil isolation, growing on oat hulls plus peptone; (2) similar inoculation with *Helminthosporium sativum*, soil isolation; (3) *Trichoderma lignorum* alone; (4) *F. culmorum* plus *T. lignorum*; (5) *H. sativum* plus *T. lignorum*; (6) all three fungi together; (7) check, no fungi added. Two pots of each were used; on November 7, 1932, ten seeds of Mindum wheat, after surface sterilization, were planted in each pot. As soon as the plants emerged it became evident that the *Fusarium* and *Helminthosporium* were injurious alone, and that *Trichoderma* alone caused no noticeable injury to the plants, and in combination with the other two fungi, rendered them harmless. Figs. 1 and 2 illustrate the condition of the plants on December 5, 1932.

FIG. 1. *From left to right: Helminthosporium sativum (soil isolation) added to sterilized soil. Second pot, H. sativum and Trichoderma lignorum. Third pot, H. sativum, Fusarium culmorum and Trichoderma. Fourth pot, check.*

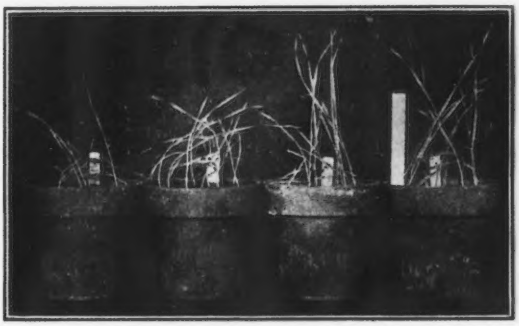

FIG. 2. *From left to right: Fusarium culmorum added to sterilized soil. Second pot, F. culmorum and Trichoderma lignorum. Third pot, T. lignorum only. Fourth pot, check.*

It is certain that *Trichoderma lignorum* is one fungus which plays an important part in reducing the danger to crops from pathogenic fungi in the soil. *T. lignorum* was found in surface soils of Manitoba to the extent of nearly 1% of the fungi present; this would indicate that each gram of surface soil harbors from 200 to 3000 viable spores or bits of mycelium of this *Trichoderma*. It is likely that *T. koningi*, certain *Penicillia*, and other organisms also may reduce the pathogenic action of soil fungi. *T. lignorum* and other species undoubtedly parasitize saprophytic fungi as well, and in turn presumably are held in check by other organisms, all of which contribute to the "balance" of the micro-organisms in a soil.

Another type of fungus-parasitism is shown by *Dactylium dendroides*, isolated once from a forest soil. This fungus is found occasionally in Manitoba parasitizing larger *Hymenomycetes* in the woods.

"Soil Fungi" Previously Found in Manitoba

Manitoba has been surveyed relatively carefully for the parasitic and saprophytic fungi found on plants or plant parts and on the ground in the fields and forests. This survey, covering about 2000 species of fungi has been published (1). When the fungus-flora of the soil is compared with that found above the soil, it becomes evident that the large majority of the soil fungi are not encountered in an ordinary mycological investigation of an area. The following only, of the fungi herein recorded from Manitoba soil, were included in the book (1) on the fungi of Manitoba:—

Mucor racemosus, common on dung.
Rhizopus nigricans, common on various substrata.
Fimetaria fimicola, on dung.
Gymnoascus reessii, occasional on dung.
Aspergillus fumigatus, on mouldy plant parts.
Aspergillus niger, sometimes appears on old plants, etc.
Botrytis cinerea, parasitizes various plants.
Dactylium dendroides, found on larger fungi.
Fusarium coeruleum, causes rot of potato tubers.
Fusarium culmorum, parasitizes cereals.
Fusarium oxysporum, in wilted potatoes.
Scopulariopsis brevicaulis, a variety on cheese.
Rhizoctonia solani, on roots of potatoes, etc.
Trichoderma lignorum, common on wood and bark.
Cladosporium herbarum, very common on old plant parts.
Helminthosporium sativum, injurious to cereal roots.

Many of the fungi found in soil, of course, would be obtained by making isolations from old leaves, debris, etc.

Does the Isolation Technique of the Soil Microbiologist give a true Picture of the Soil Fungi?

The methods of counting soil fungi have been standardized fairly well (3) but there are various objections to these methods, as expressed by Brierley *et al.* (2, 3), Thom (21), and others. Attempts have been made to obtain other perspectives by Winogradsky's direct microscopic examination of soil, and by Cholodny's technique in which microscope slides are placed in the unmolested soil for one to three weeks before examination. Isolation methods, however, have the advantage of yielding cultures which usually are determinable.

Since spores of fungi fall upon the soil from all sources, one might expect to isolate from soil almost any fungus that grows readily on common agar media. It must be remembered, however, that the true soil fungi, *i.e.*, those which live and reproduce in the soil, will be isolated nearly always in larger numbers than those fungi which have grown elsewhere. Furthermore, enough is known of the common fungi to make it possible usually to estimate the

source of the fungus once its name is determined. If a fungus is present to the extent of 1% or more of the total number isolated, it is safe generally to conclude that it is an active soil fungus.

Many important soil fungi however, are rarely or never obtained by the ordinary isolation technique. The mycelium of *Hymenomycetes*, for example, unquestionably plays an important part in the disintegration of organic matter in soils, particularly forest soils, and spores of these fungi fall in great numbers upon the ground. Since the larger fungi are often heterothallic, however, cultures obtained seldom are identifiable even to group. Isolation technique needs to be supplemented by field study to gain a proper idea of the soil fungi.

Fungi which grow slowly on culture media usually are swamped on ordinary isolation plates, but a number of cultures of such fungi may be obtained by examination of plates from higher dilutions held longer. Possibly a few fungi of some importance in soil will not grow on common agar media.

An analysis of the data presented in this paper indicates that the isolation methods used give a picture of the activities of soil fungi which must be true in many respects. *Mucorales*, *Penicillia*, *Trichoderma* and other saprophytes known to be important in breaking down organic material have been obtained abundantly. Smaller counts of the parasitic fungi known to exist in field soils were found. *Trichoderma* is important in preventing the parasites from dominating the soil. A number of common fungi likely to be present accidentally in surface soils have been found there in relatively small numbers. Isolation methods give consistent counts showing that fungi are more common in forest than in field soils, and that they may increase when fertilizer is added. They show that the fungi are only dormant in a cold winter.

Care must be taken in soil isolation work to preclude laboratory contaminations in the plates counted. It is almost inevitable that an occasional spore from the air may enter and the resulting colony be counted as a soil fungus. This source of error tends to raise the count of *Penicillia* in particular, but can be eliminated to practical insignificance.

Variation in Soil Fungi

After examining a few thousand cultures of fungi, one is impressed by the variation many of them show. These may be classified as morphological variations, represented by slight differences in size, shape, etc., of spores, in characters of conidiophores, or in type of growth on culture media; and physiological variations shown in color changes produced in the media, or variations in odors, etc. For example, *Penicillium terrestre* often has a very characteristic fruity odor; but more often it is lacking in cultures morphologically indistinguishable. While some of these variations might be worthy of variety or form names, in many cases there are intergradations which would make a varietal name useless or confusing. Certain groups of fungi, such as *Trichodermas* and *Cephalosporiums*, need critical revision; but most soil fungi can be identified, if one allows something for the variations that occur.

The Distribution of Soil Fungi

Many of the fungi found in Manitoba soils have a very wide distribution over the world. Miss Dale (4, 5) found 57 species of fungi in soil in England, more than one-third of which have been found in Manitoba soil. Gilman and Abbott (9) give distribution records from which it is evident that many soil fungi have a wide distribution. Brierley (2) considers that *Aspergilli* may be more common in warmer temperate regions, and Miss Todd (22) reports, as mentioned above, high counts of *A. niger* in Oklahoma. *Aspergilli* are not very abundant in Manitoba soils.

Critical comparisons of the distribution of soil fungi are not possible until more data are available. LeClerg (14) has analyzed 7000 isolations from Colorado soils, 6880 of which he includes under 31 names. He also found *Penicillia* to be the commonest soil fungi, but found a higher proportion of *Aspergilli* than have been found in Manitoba.

There are definite differences in the fungus-flora of distinct soil types, or from the same type of soil cropped to different plants, but the work here reported has not progressed far enough to make clear the significant differences, nor to explain the variations in number or types of fungi found in the soil during the four seasons. It is believed, however, that sufficient knowledge of the fungi in surface soils in Manitoba has been gained to make possible further results of practical value from an intensive study of certain phases of their activity.

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THE EFFECT OF MECHANICAL SEED INJURY ON THE DEVELOPMENT OF FOOT ROT IN CEREALS[1]

By J. E. Machacek[2] and F. J. Greaney[3]

Abstract

Greenhouse and field experiments have shown that the use of mechanically injured seed promotes the development of seedling blight and foot rot caused by *Fusarium culmorum* in cereals, thereby retarding the growth of the plants and decreasing yield.

The presence of a large proportion of broken or bruised kernels in seed grain seriously reduces its market value. A secondary loss, caused by invasion of seed grain by fungi after the seed is sown, also occurs but very few experimental data regarding this loss are available.

It is known that fruits and vegetables are protected from the invasion of many rot-inducing micro-organisms by cuticular and epidermal layers, or waxy exudations known as "bloom". Seeds of the *Gramineae* are similarly protected. Brown (1) observed that a semipermeable membrane covers the seed, making it somewhat impermeable to toxic salts. It has been found by other investigators that the permeability of the testa is increased when the tannin content of the testa is low (11, 12), when the testa is undeveloped owing to drought (3), and when the seed is unripe or damaged (6). Nobbe (9), according to Hurd (5), found that machine-threshing injured the seed coat sufficiently to permit chemical injury in the seed after treatment with copper sulphate. Walldén (13), according to Hurd (5), confirmed Nobbe's findings, and was able to correlate the degree of chemical injury with the amount of damage to the seed coat. Pugh and others (10) found that the testa of wheat kernels becomes increasingly resistant to penetration by *Gibberella saubinetii* (Mont.) Sacc. as the grain matures. Hurd (5) found that chemical or mechanical seed injury predisposes the seed to attack by saprophytic fungi. Walldén (13) also reported marked susceptibility to moulds in mechanically injured seed when the grain was stored under improper conditions.

The data presented here show that mechanical injury predisposes seed grain to infection by fungi, particularly *Fusarium culmorum* (W. G. Sm.) Sacc., and consequently, in infested soil, seedlings arising from injured seed are more frequently diseased than seedlings from uninjured seed.

Materials and Methods

Several varieties of wheat and a hull-less variety of oats and of barley were used in these experiments. The seed coat was injured by one of three methods: (*a*) puncturing slightly the embryo end, (*b*) clipping off a small portion of the "brush" end, or (*c*) scarifying the seed lightly with coarse sandpaper. The effect of each type of injury was determined by planting the seed in autoclaved soil to which a quantity of *F. culmorum* inoculum was added. The inoculum consisted of a fresh culture of the fungus grown on an autoclaved, ground oat-hull medium.

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Contribution from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.
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Experimental Results

Greenhouse Experiments

In the majority of the greenhouse experiments only punctured or clipped seed was used. The inoculum (one part inoculum to nine parts soil, by volume) was added to autoclaved soil, and both were mixed by means of a drum mixing machine. In each test four six-inch pots, each containing 25 seeds planted at a depth of 1 in., were used for each type of seed injury. Checks with uninfested autoclaved soil were used. The pots were kept at approximately 20° C. and watered daily.

Notes on seedling emergence and disease prevalence were taken two weeks after planting. It was observed that wherever injured seed, as compared with uninjured seed, was planted in infested soil, reduced emergence and increased foot rot occurred. In calculating the percentages of foot-rotted plants only the emergent seedlings were considered. The results are illustrated graphically in Figs. 1 and 2, and summarized in Tables I and II.

Field Experiments

These experiments were designed to determine the effect of mechanical seed injury on foot-rot development under field conditions. Healthy and scarified seed of Marquis and Mindum wheats were used. The complete field experiment in 1932 consisted of a series

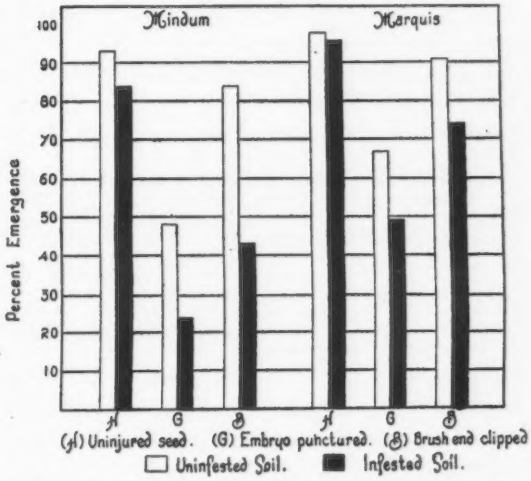

FIG. 1. *The effect of mechanical seed injury on the incidence and severity of Fusarium culmorum infection in the greenhouse. A. Seedling emergence.*

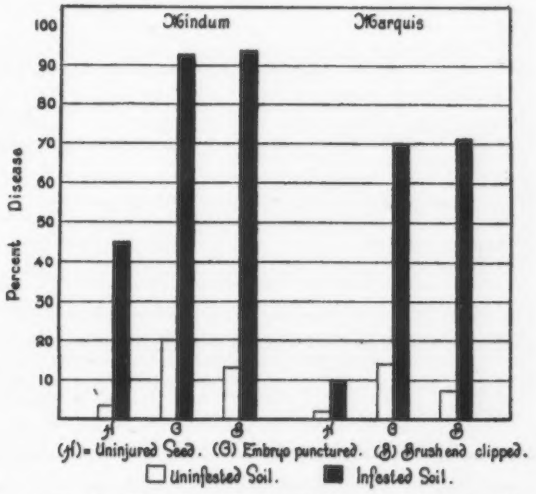

FIG. 2. *The effect of mechanical seed injury on the incidence and severity of Fusarium culmorum infection in the greenhouse. B. Percentage disease in emergent seedlings.*

of eight blocks, each block composed of eight plots. Each plot consisted of two rod-rows, one in which the seed was spaced, 100 to the row, and the other containing 15 gm. of Marquis or 18 gm. of Mindum seed sown by hand and distributed evenly in the row. The inoculum, 600 cc. (1 : 9 mixture, as used in the greenhouse, but incubated for two weeks) per rod-row, was distributed evenly at seed level through each row to be infested. There was complete randomization of varieties (Marquis and Mindum), seed treatments (injured and uninjured), and soil treatments (infested and uninfested) within each block.

TABLE I

THE EFFECT OF MECHANICAL SEED INJURY ON THE AVERAGE EMERGENCE OF SOME CEREAL VARIETIES GROWN IN THE GREENHOUSE IN UNINFESTED SOIL AND SOIL INFESTED WITH *Fusarium culmorum*

Variety	Percentage of emergence			Mean
	Uninjured seed	Embryo punctured	Brush end clipped	
Marquis (wheat)	97.0	57.5	82.2	78.9
Mindum (wheat)	88.7	38.0	63.2	60.0
Pen. x Ma. 729 (wheat)	63.0	38.5	67.0	56.1
H-44 x Ma. 586 (wheat)	78.0	49.5	83.0	70.1
Laurel (oats)	70.7	64.0	69.0	67.9
Guy Mayle (barley)	69.0	46.7	67.0	60.9
Mean	79.7	49.2	71.3	

TABLE II

THE EFFECT OF MECHANICAL SEED INJURY ON SEEDLING EMERGENCE AND SEVERITY OF *Fusarium culmorum* FOOT ROT IN THE GREENHOUSE

Treatment	Uninjured seed	Embryo punctured	Brush end clipped	Mean
A. Average emergence in six cereal varieties, per cent				
Uninfested soil	95.1	71.8	91.2	82.7
Infested soil	63.3	26.7	51.4	47.1
Mean	79.7	49.2	71.3	
B. Percentage foot rot in emergent seedlings				
Uninfested soil	12.9	14.3	13.4	13.5
Infested soil	41.6	84.6	78.0	68.1
Mean	27.2	49.4	45.7	

Approximately ten days before harvest, the plants in the spaced rows were pulled and examined individually for the purpose of obtaining a disease rating based on the presence or absence of basal lesions indicating foot rot.

TABLE III

THE EFFECT OF MECHANICAL SEED INJURY ON THE INCIDENCE OF *Fusarium culmorum* FOOT ROT OF WHEAT IN FIELD PLOTS

A. Soil treatments and disease rating

Treatment	Disease rating		Mean	
	Uninjured seed	Sand-papered seed		
Uninfested soil	66.1	68.2	67.2	Standard error
Infested soil	72.1	77.4	74.7	0.90
Mean	69.1	72.8		
Standard error 0.71				

B. Varieties and disease rating

Variety	Disease rating		Mean	
	Uninjured seed	Sand-papered seed		
Marquis	65.8	69.3	67.6	Standard error
Mindum	72.4	76.4	74.4	1.26
Mean	69.1	72.8		
Standard error 0.71				

C. Soil treatments and yield

Treatment	Yield in bushels		Mean	
	Uninjured seed	Sand-papered seed		
Uninfested soil	38.9	35.7	37.3	Standard error
Infested soil	33.4	29.7	31.5	0.96
Mean	36.1	32.7		
Standard error 1.07				

D. Varieties and yield

Variety	Yield in bushels		Mean	
	Uninjured seed	Sand-papered seed		
Marquis	28.9	25.0	27.0	Standard error
Mindum	43.4	40.5	41.9	1.91
Mean	36.1	32.7		
Standard error 1.07				

From the data so obtained a disease rating was calculated for each plot. A modification of the method described by McKinney (8) was used. The second row in each plot furnished the yield data for the plot.

The analysis of variance method, as devised by Fisher (4), was used in analyzing the disease-rating and yield data. The effect of soil treatments and of varieties on disease rating in plants grown from injured and uninjured seed and the effect of the same factors on yields from injured and uninjured seed plots are shown in Table III. It will be observed that significant increases in disease rating and significant decreases in yield resulted from seed injury. The wheat variety Mindum appeared more susceptible to *F. culmorum* foot rot than the variety Marquis. In the final analysis of the experimental results, disease ratings and yields of individual plots were correlated, and a highly significant negative coefficient (−.5129) was obtained.

Discussion

The mechanical injury of seed appears to be an important factor in the development of pathologic conditions other than the foot rot caused by *F. culmorum*. The investigations of Hurd (5) and Walldén (13) show that mechanically injured seed is particularly susceptible to invasion by saprophytic fungi. Hurd found that mechanical injury lowers seed vitality, although germination may be unimpaired. If favorable conditions are present, the injured seed, though its initial germination is good, soon becomes invaded by saprophytic fungi, the invasion resulting in distortion and stunting of the seedlings, and often in lesioning of the coleoptile and primary roots. Hurd believes that the seed testa protects the seed from fungous invasion, and that, if the testa is broken, the nutrients contained in the endosperm become readily available to these fungi, resulting in their luxuriant growth when conditions of temperature and moisture permit. Consequently, according to her findings, mechanical seed injury, as well as other factors that lower vitality of seed grain, predisposes the seed to invasion by fungi.

It is probable that the same host-parasite relationship exists in the development of *F. culmorum* foot rot in cereals. Through mechanical injury of the seed the endospermic nutrients become readily available to the fungus, thereby promoting its growth, but at the expense of seed and seedling. That this theory is at least partly correct is shown by the examination of the greenhouse experimental data. A considerable number of seedlings failed to reach the soil surface when injured seed was planted in infested soil, whereas uninjured seed produced seedlings that, under similar conditions, were able to emerge from the soil in large numbers. The possible increased growth of the fungus, in the case of the injured seed, is directly reflected in the rapid development of pre-emergence blight. It would therefore seem probable that the use of uninjured seed would be advantageous to the farmer in his attempt to produce a disease-free crop.

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ON THE SPECIFIC HEATS OF TUNGSTEN, MOLYBDENUM, AND COPPER[1]

By H. L. Bronson[2], H. M. Chisholm[3] and S. M. Dockerty[3]

Abstract

This paper contains the results of a long series of determinations of the specific heats of tungsten, molybdenum, and copper from −20° to 500° C.

A new type of all-copper adiabatic calorimeter has been designed and used. The complete elimination of water from the calorimeter removed several sources of error and resulted in increased reliability and accuracy.

Two entirely different methods were used in determining the specific heats. The usual "method of mixtures" was used to determine the mean specific heat for a large temperature change and was applied to all three metals over the entire range of temperature. The specific heat of copper was also determined for 5- or 10-degree intervals from −5° to 110° C. by heating the calorimeter electrically.

It has been quite definitely shown that the specific heats of these metals over a temperature range as large as 0° to 500° C. cannot be expressed as a linear function of the temperature. An equation of the form $C_p = A + BT - C/T^2$ was arrived at from theoretical considerations and the constants determined empirically with the following results:—

Tungsten..... $C_p = 0.03199 + 0.00000328T - 129/T^2$

Molybdenum..... $C_p = 0.06069 + 0.0000120T - 361/T^2$

Copper..... $C_p = 0.09292 + 0.0000136T - 452/T^2$

where the unit of heat is the 20-degree calorie and T is absolute temperature. The average deviation of the individual determinations from the values calculated by these equations was only about 0.1%.

As a matter of convenience and for purposes of comparison, linear equations applicable over smaller ranges of temperature have also been given.

Introduction

Many problems both theoretical and practical require for their solution reliable and accurate values of the specific heats of the metals. An examination of the specific heat data given in the International Critical Tables (I.C.T.) shows that such values are not available. Most of the data are old and there are wide discrepancies among the results of different observers. Unfortunately, however, wide discrepancies are not confined to the older work. For example, at the time that this investigation was started, two of the most recent and presumably reliable values (7, 9) for the specific heat of tungsten differed by about 30%.

The value obtained for the specific heat of a body by the usual calorimetric methods is the mean value for a considerable change of temperature. The final temperature is most commonly about room temperature and the initial temperature 100° C. or higher. For some of the metals one such mean value over a single temperature range is all that is given in the I.C.T. This is entirely unsatisfactory where even a moderate degree of accuracy is required, since the change in specific heat from 0° to 100° C. may be as much as 5%.

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In the cases of most of the more common metals for which more data are available the I.C.T. combines all the information into "best" values and expresses the specific heat as a linear function of the temperature. It is difficult to estimate the accuracy of the values given but an uncertainty in some cases of as much as 50% in the value given for the temperature coefficient of specific heat would seem quite possible. In fact, two values are given in the case of aluminium which differ by more than this.

The results of the present investigation show very conclusively that the temperature coefficients of specific heat of tungsten, molybdenum, and copper are not constant but decrease with increasing temperature, the average value between 0° and 150° C. being about double that between 150° and 500° C. The *rate of decrease*, however, diminishes rapidly with increasing temperature becoming at 500° less than 2% of its value of 0° C. These facts probably account for many of the discordant results obtained by previous experimenters.

If the change in the temperature coefficient is to be detected and measured, either the mean specific heat for large changes of temperature must be determined with greater accuracy than has been usual in this type of work, or some way must be found of obtaining the actual specific heat at a given temperature, that is, over a small range, for a considerable number of quite different temperatures. Both methods have been employed in the present investigation, with entirely consistent results.

The specific heat of copper is probably known with at least as great accuracy as that of any of the metals. There are, however, discrepancies among the most reliable data which it seems desirable to point out and discuss briefly even though the results of this investigation are anticipated in so doing.

The work of Harper (5) at the Bureau of Standards is a striking exception to the usual practice of measuring the mean specific heat for a large change in temperature. He determined the specific heat of copper for two-degree intervals from 0° to 50° C. and obtained the following linear equation which should be trustworthy for the range given:

$$C_p = 0.0905 + 0.000048t,$$

where C_p is the specific heat at constant pressure in cal./gm.°C. and t is in °C.

A comparison of this equation with another of the same form but for a larger range of temperature shows that the specific heat curve is actually far from linear. Since the "best" values given in the I.C.T. should be as reliable as any available, they were used and the following equation obtained for the range 0° to 500° C.:

$$C_p = 0.0916 + 0.000025t.$$

These two equations give the same value for C_p at about 50° C. and the constant terms differ by about 1%, but the interesting fact is that the temperature coefficient of the first is nearly double that of the second.

In the present investigation the values obtained for the specific heat of copper between 0° and 50° C. differ from those obtained by Harper only by

0.15%. Our experiments, however, were extended to include temperatures up to 500° C. and the temperature coefficient was found to change from about 0.000058 at 0° to 0.000048 at 25°, to 0.000040 at 50°, to 0.000031 at 100°, to 0.000020 at 250°, and to 0.000016 at 500° C., the last value being even smaller than that given in the I.C.T.

It has been quite commonly assumed, for example in the I.C.T., that the relation between specific heat and temperature could be expressed by an equation of the form

$$C_p = A + Bt + Ct^2.$$

In fact, only occasionally has an attempt been made to express experimental results in any form other than a first or second degree equation. That even a second degree equation is quite unsuitable for the purpose is indicated by the above values for the temperature coefficient of copper, which show that the *rate of change* of the temperature coefficient with the temperature decreases rapidly and approaches zero at high temperatures, while it is constant in a second degree equation.

General Method

An adiabatic calorimeter of the Richards' type was used throughout this investigation, but two entirely different methods were employed in the determination of the specific heats. In the first, the specimen under investigation was heated in a suitable electric furnace and allowed to fall directly into the adiabatic calorimeter and its specific heat was calculated as usual in the method of mixtures. Two types of calorimeter were used, the usual one containing water and another where the water was replaced by an all-copper calorimeter. The water equivalent of each calorimeter was determined by heating it electrically and measuring the energy input per degree rise in temperature. The equivalent of the water calorimeter was also calculated in the usual way, using the masses and specific heats of its various parts. By this first method were obtained the *mean* specific heats of tungsten, molybdenum, and copper between room temperature and various other temperatures from −20° to 500° C.

In the second method the solid copper calorimeter was heated electrically from below 0° to above 100° C. The energy supplied per gram per degree rise in temperature was determined for successive small temperature intervals. From this the actual specific heat of copper at a definite temperature was readily calculated. This method was more accurate than the first, but could not be applied to tungsten and molybdenum as sufficient material in suitable form was not available.

An effort was made in this work to discover and eliminate those constant and unsuspected sources of error which have caused such wide discrepancies among the results of different investigators. To this end the apparatus and the methods have been continually altered. The writers have aimed at reliability rather than high precision, but even so it is felt that a higher precision than is usual in this type of work has been attained.

Apparatus and Experimental Methods

The Adiabatic Calorimeter

This consisted essentially of the calorimeter, *C*, Fig. 1, the bomb or "jacket", *B*, and, surrounding *B*, a water bath, which is not shown in the figure. The temperature of the calorimeter was measured by a Beckmann or a platinum thermometer in the outside water bath which was kept in thermal equilibrium with the inside calorimeter. The equality in temperature between the two was indicated by a sensitive differential thermocouple. Under these circumstances there should be no heat transfer between the calorimeter and surrounding bodies. However, instead of the temperature of the calorimeter remaining constant, there usually occurred a slow change or "creep" in its temperature which necessitated the making of uncertain and time-consuming corrections, occasionally as large as 2%. This "creep" was quite erratic, being sometimes positive and sometimes negative, and might have been due to a number of causes; *e.g.*, evaporation, stirring, or failure of the differential thermocouple to indicate exact equality of temperature because of non-homogeneous constantan wire.

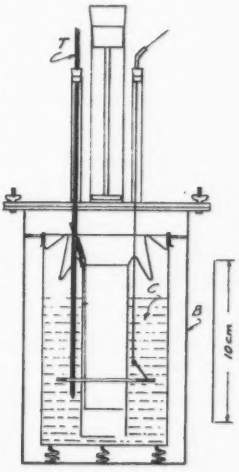

FIG. 1. *Water calorimeter and jacket.*

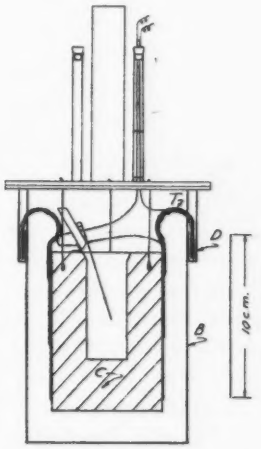

FIG. 2. *All-copper calorimeter and jacket.*

The calorimeter shown in Fig. 1 was of the usual type, and consisted of a nickel-plated copper beaker of about 500 cc. capacity. Within this was another smaller copper vessel having a cover arranged to close automatically as the specimen entered and thus prevent heat losses by convection. The total mass of copper in this calorimeter was about 200 gm. and from 150 to 400 gm. of water was used in the various experiments. Suitable stirring was obtained by a copper ring which was raised and lowered about 30 times per minute. This stirrer was operated very simply and satisfactorily by means of a worm and wheel attached to one of the motors used in stirring the outside water bath.

The all-copper calorimeter shown in Fig. 2 proved to be a great improvement over the water calorimeter and was used in all the later experiments. With it there was no troublesome water vapor and no stirring. There was the added advantage that the differential thermocouple wires, *T*, were entirely enclosed in a constant-temperature region, instead of having to pass outside the jacket as in Fig. 1. For this reason, probably, the all-copper calorimeters

were practically free from the "creep" which proved so troublesome with the water calorimeters.

Three of these all-copper calorimeters, of masses about 1, 3, and 5 kgm., were made in order that the temperature changes in the various experiments might not be too large to measure with a 5°-range Beckmann thermometer and yet not too small for the desired precision. Each calorimeter had a 2.5-cm. hole drilled as shown and a heavy copper cover arranged to close automatically. Castings for the two larger calorimeters were made by Tallman Brass and Metal Ltd., of Hamilton, Ont., and were said to be of 100% pure copper. The smallest was made from a piece of especially pure 2-in. copper rod supplied by the Halifax Shipyards. The densities of the three were respectively, 8.82, 8.81, and 8.90 gm. per cc. The same jacket was used with all the calorimeters. It was of nickel-plated brass and had a capacity of about 1100 cc.

Water and Oil Baths

The water bath surrounding the jacket was equipped with hot and cold water connections and could be heated electrically as well. After a broken belt had cracked the case of one Beckmann thermometer, the usual stirring paddles with belts and pulleys were replaced by two $\frac{1}{80}$-h.p. high-speed motors direct-connected by 8-in. extension shafts to the stirring paddles which operated inside short lengths of 2-in. pipe. The motors were fastened to the sides of the tank with shafts nearly vertical. This arrangement was much safer and more convenient than the belts and pulleys usually used. A careful examination with a platinum thermometer showed no variation of over 0.001° in the temperature of the water around the jacket even when it was 25° C. above room temperature.

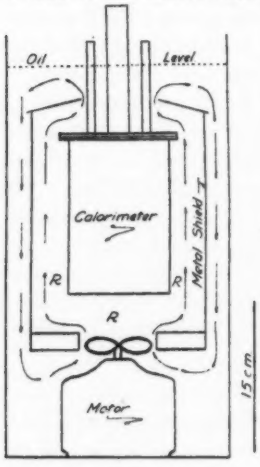

FIG. 3. *Oil bath and calorimeter.*

In some experiments to be described later, a bath was required which could be operated from temperatures below 0° to above 100° C. A design that proved very satisfactory is shown in Fig. 3. Kerosene was used for the lower temperatures and transformer oil for the higher. A small fan motor with blades cut short was mounted in the oil as shown and produced good circulation. The top of the bath was well covered with cotton wadding and the outside wrapped with asbestos paper. It was then packed in sawdust or a freezing mixture as required. The temperature was controlled by electric heating and by hot or cold water passed through a coil of copper tubing. All the heating or cooling took place in the space outside the metal shield and the oil was thoroughly mixed by the fan before reaching the calorimeter jacket. The space *R* was thus completely shielded from outside disturbances and the temperature gradient at the boundary was small.

Differential Thermocouples

Accurate temperature equality between the calorimeter and surrounding jacket was assured by means of a six-junction differential thermocouple of No. 34 copper and No. 32 constantan wire connected directly to a sensitive galvanometer which gave about 5 mm. deflection for a temperature difference of 0.001° C. In the case of the water calorimeter, the junctions were mounted in thin-walled glass tubes which were placed in the water of the calorimeter and of the bath.

In the earlier experiments with the copper calorimeter, one set of six junctions was distributed in two slots on opposite sides of the copper calorimeter and the other six junctions were placed three in each of two small oil-filled cavities (*D*, Fig. 2), on opposite sides of the jacket.

In both the above types of thermocouple, considerable uncertainty existed regarding the relative lag in temperature of the junctions behind that of the jacket and of the calorimeter, when the temperatures of the two were rising at the same rate. In order to eliminate this uncertainty, a completely symmetrical arrangement of the thermojunctions was used in the latter part of this work. Two three-junction couples were used in series and each of the four sets of three junctions was similarly mounted in a short piece of thin $\frac{1}{8}$-in. brass tubing.*

Two vertical $\frac{1}{8}$-in. holes about 2-in. deep were drilled close to the surface on opposite sides of each copper calorimeter and two pieces of $\frac{1}{8}$-in. copper tubing were soldered to the jacket. The junctions fitted neatly in these holes and could be easily removed and replaced without damage. Each thermocouple was tested by placing its junctions in the two holes of one copper calorimeter and then heating it. Exact similarity of the junctions was indicated if the galvanometer gave no deflection.

Measurement of Bath Temperature

During the earlier part of this work the temperature of the bath was measured with a standard Beckmann thermometer with Reichsanstalt certificate. Some question having arisen about its reliability, a comparison was made between it and two other standard Beckmann thermometers (also with Reichsanstalt certificates) and also another standard thermometer with Bureau of Standards certificate. No two of these thermometers agreed in the measurement of a temperature difference. In fact, the two in closest agreement differed by about 0.015° in a 5° interval. The writers were not satisfied with this degree of accuracy and reluctantly decided to make their own temperature calibrations.

A platinum thermometer bridge of the Callendar-Barnes type (2) was built and calibrated. The bridge was so made that a one-degree change in a 20-ohm platinum thermometer corresponded to about 20 cm. of bridge wire.

**Johns-Manville Plastic Refractory Cement No. 20 proved to be satisfactory for holding and insulating the junctions in the tubes. It was suitable for all temperatures and when thoroughly dry the insulation between couple and case was over 10^8 ohms.*

The sensitivity of the galvanometer was such that there was no difficulty in making settings to 0.1 mm. or 0.0005° when the current through the thermometer was 0.005 amp.

The bridge wire of manganin was carefully drawn and tested for uniformity. No 4-cm. section was found to differ by as much as 0.1 mm. from any other 4-cm. section. The bridge coils of manganin had a negative temperature coefficient of about 0.000005 at room temperature and the larger ones were approximately compensated by copper wire in series. This compensation was quite unnecessary as far as this investigation was concerned for the coils were in an oil bath the temperature of which was kept sufficiently constant to avoid errors. Repeated calibrations of this bridge showed the coils to be constant to within 0.2 mm. of bridge wire.

Two platinum thermometers of about 20 ohms each were made. The first was from commercial platinum which proved to have a temperature coefficient of 0.00345 and $\delta = 1.59$. The second was of c.p. platinum having a temperature coefficient of 0.00391 and $\delta = 1.52$. Repeated determinations of the fundamental intervals of these thermometers indicated that they were reliable to within 0.01%.

These two thermometers were in good agreement in their measurements of temperature differences between 0° and 100° C. Both gave the same corrections for two of the Beckmann thermometers previously mentioned and showed that one read too high and the other too low by about 0.008° in 5° C. All the later measurements of the bath temperature were made with one or the other platinum thermometer and the writers have found no reason for questioning the reliability of these measurements. The earlier measurements made with the Beckmann were all corrected by the platinum thermometer calibration.

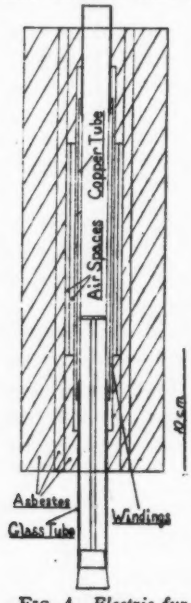

FIG. 4. *Electric furnace for heating specimens.*

Furnace

A fairly satisfactory furnace for heating the specimen was made by using a 10-in. length of ¾-in. heavy copper pipe with about 3-in. of glass tubing of the same internal diameter fitted to each end (Fig. 4). Wire and insulating material were wound on this and adjusted with extra turns of wire at the end until there was an 8-cm. space at the centre which did not show any temperature variation of over 0.2° at 300° C. when explored by a thermocouple, even when the specimen was not present. The resistance of the heating coil was about 130 ohms, and 0.6 amp. raised the temperature to about 325° C.

Cooler

In order to get specific heats below room temperature, a simple "cooler" was made by passing a ¾-in.

brass tube through the centre of a can 6 in. in diameter and 12 in. long in which was placed ice or a freezing mixture. The specimen was suspended in the centre of the tube and could be dropped directly into the calorimeter in the same way as from the furnace.

Measurement of Furnace Temperature

The temperature of the metal specimen in the furnace or cooler was obtained by means of a single-junction thermocouple which was inserted in a hole drilled in the specimen. These holes extended practically the entire length of the copper and molybdenum specimens, but the writers were unable to drill more than 5 mm. into the tungsten even with a diamond drill. In some of the experiments at the higher temperatures the tungsten was surrounded by a copper shield which came well up on the thermocouple thus ensuring that it was at the temperature of the tungsten, but this produced no noticeable change in the results.

The e.m.f. of the thermocouple was measured in the usual way on a Leeds and Northrop type K potentiometer. The standard cell was kept in a thermostat and its e.m.f. was known by comparison with standard cells at McGill University.

Temperatures up to 350° C. could be satisfactorily measured with a couple of No. 34 copper and No. 32 carefully selected commercial constantan wire, and such couples were used in all the earlier experiments. The junctions were placed in thin-walled glass tubes, one of which was always in a large Dewar flask filled with ice. The couples were calibrated in steam and in melting tin, cadmium, and zinc. The metals were melted in cylinders of iron or carbon which just fitted into the furnace used for heating the specimens. Thus during calibration the couples were under exactly the same conditions as when used in measuring temperature. Another advantage of this arrangement was that the furnace gave such perfect control of the rate of cooling that at the melting point the temperature would not change by as much as 0.05° C. in 10 min.

The thermoelectric properties of pieces of commercial constantan wire even from the same spool differ considerably. Curve *A*, Fig. 5, is the calibration curve of one of these couples. The method suggested by Adams (1) has been used. The abscissas give the values of the e.m.f. in microvolts, and the ordinates the differences between these values and

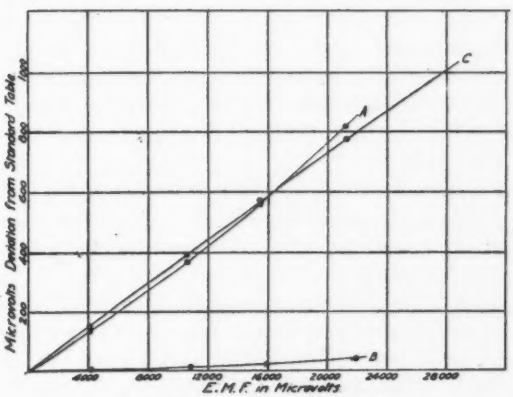

FIG. 5. *Thermocouple calibration curves.*

those given by Adams for the same temperatures. By the use of this deviation curve the temperature corresponding to any e.m.f. is readily obtained from the equation given by Adams or, more conveniently, from his very accurately calculated Calibration Table. Later it was possible to check these results with a couple made from special high grade constantan wire which was very kindly supplied by L. H. Adams of the Geophysical Laboratory at Washington. This wire was from the same lot as that used by him in determining his thermocouple calibration tables and equation (1). The close agreement of our calibration of this thermocouple with that given by Adams is shown in Table I and by curve *B*, Fig. 5, and confirms the

TABLE I

COMPARISON OF CALIBRATIONS OF THE HIGH GRADE CONSTANTAN COUPLE (FIG. 5, CURVE *B*)

Temperature, °C.	100	231.8	320.9	419.4
E.m.f. by Adams, microvolts	4276	11009	16083	22075
E.m.f. by authors	4274	10993	16055	22027
Difference	2	16	28	48

accuracy of our methods of calibration. The deviations of both thermocouples from the Standard Table are due to slight differences in the constantan alloy, but thermocouple *B* has two great advantages over *A*. In the first place, its deviation curve could be drawn to a larger scale, thus increasing the accuracy of the temperature determination, and in the second place, there was no danger of disturbing effects due to lack of homogeneity which are always possible when commercial constantan is used.

Since copper-constantan couples of fine wire deteriorate rapidly above 350° C., couples of platinum and platinum with 10% of rhodium and couples of alumel-chromel were tried. The latter were used in a large number of experiments, but all of these were finally rejected. Lack of homogeneity in the wires was apparently responsible for errors of as much as 1%.

It was finally decided to try at higher temperatures a couple of No. 30 copper and No. 28 constantan, also kindly supplied by Mr. Adams. This couple proved quite satisfactory up to 500° C. Its calibration curve taken after its use at this temperature differed by less than 0.1% from that taken before. Its deviation curve extrapolated to 500° C. is shown as *C*, Fig. 5. Adams' Calibration Table extending from 0° to 385° C. was calculated from the equation

$$E = 74.672t - 13892\ (1 - e^{-0.00261t}).$$

In order to test the applicability of this equation up to 500° C., one junction of the couple and the platinum thermometer were placed close together in holes drilled in a piece of $\frac{3}{4}$-in. copper rod. This was placed in the centre of the furnace and its temperature measured by both the thermocouple and the platinum thermometer at four points, the lowest being about the melting point of zinc at which temperature the e.m.f. of the couple was known. The result of this comparison is shown in Table II, and quite justifies the use of the couple for measurements up to 500° C.

TABLE II

	1	2	3	4
T, by platinum thermometer, °C.	418.8	445.5	471.1	501.3
T, by thermocouple, °C.	418.8	445.6	471.3	501.4

Specimens of Copper, Molybdenum, and Tungsten

All the specimens were in the form of cylinders from 1.5 to 1.7 cm. in diameter and from 2 to 4 cm. in length. Four specimens of masses about 34, 51, 69, and 89 gm. were made from commercially pure cold-rolled copper rod of density about 8.90 gm. per cc. The two specimens used at high temperatures were nickel-plated to prevent oxidization. The small amount of nickel deposited was known and corrected for when calculating the specific heat.

The writers had at their disposal only one cylinder of molybdenum and two of tungsten. All three were supplied through the kindness of the Research Laboratory of the General Electric Company and were supposed to be pure.

The specific heat of the molybdenum cylinder had previously been determined by Cooper and Langstroth (3). It had a mass of about 70 gm. and specific gravity of about 9.75. One cylinder of tungsten, of mass about 133 gm. and specific gravity 19.05, had been carefully swaged and polished. The other tungsten specimen, of mass about 83 gm. and specific gravity 17.3, was made from a block that had not been as carefully swaged and was not nearly as hard. In fact, a hole for the thermocouple was successfully drilled in it. The specific heat of this specimen seemed continually to increase when heated above 400° C. Finally it split open, revealing inside a considerable amount of greenish-yellow porous material, probably an oxide, which doubtless accounted for its low density and increasing specific heat. The results obtained with this specimen were therefore rejected.

Slight traces of oxidization were evident on the surfaces of the tungsten and molybdenum specimens, but not enough to cause a change in weight of 0.01 gm.

Thermal Capacity of Water Calorimeter

The water equivalent or thermal capacity of the water calorimeter was determined by two different methods. The first consisted merely in adding to the weight of water the water equivalent of the component parts of the calorimeter determined as follows:

	Mass, gm.	Sp. ht.	Water equiv., gm.
Copper calorimeter and stirrer	197.3	0.0915	18.05
Glass thermocouple tube	1.7	0.16	0.27
Thermocouple wire	1.0	0.097	0.10
Thermocouple cotton insulation	0.3	0.4	0.12
Paraffin wax	0.1	0.7	0.07
Air heated by calorimeter			0.06
Cooling due to evaporation			0.4
Total			19.1

In the second method the total thermal capacity in joules per °C. was determined electrically. About 60 ohms of cotton-covered manganin wire was wound on a thin copper cylinder and placed in the inside calorimeter vessel. Copper lead wires were brought out through holes in the cover and wound several times around the outside of the calorimeter to prevent any possible conduction of heat from the coil out to the jacket. Potential leads were soldered to these current leads just above the calorimeter and the four leads carried out through one of the tubes in the jacket cover. The current and potential were measured by standard methods, using a Leeds and Northrop type K potentiometer and standard cell. All resistances used were carefully calibrated. The current was adjusted to give a rise in temperature of about 0.25° per min. and the outside bath was heated electrically at the same rate, the differential thermocouple being kept balanced to 0.01°. The time, usually 15 or 20 min., was easily measured with a good watch to 0.2 or 0.3 sec.

The corresponding water equivalent was obtained by using the relations

$$1 \text{ calorie}_{15} = 4.1835 \text{ electrical joules}$$
$$1 \text{ calorie}_{20} = 4.180 \text{ electrical joules}$$
$$1 \text{ calorie}_{25} = 4.176 \text{ electrical joules}$$

From the result thus obtained it was necessary, in order to get the water equivalent of the calorimeter, to subtract the mass of water used and the water equivalent of the heating coil. The mass of water used was between 300 and 350 gm. The water equivalent of the heating coil was small and was obtained with sufficient accuracy as follows:

	Mass, gm.	Sp. ht.	Water equiv., gm.
Copper cylinder	15.37	0.0915	1.41
Manganin wire	2.54	0.097	0.25
Cotton insulation	0.97	0.4	0.39
Copper lead wire	0.37	0.09	0.03
Cotton insulation	0.07	0.4	0.03
Total			2.1

The mean of three such determinations of the water equivalent of the calorimeter was 19.3 gm. This is in better agreement with the previous value (19.1 gm.) than might be expected, the difference, 0.2 gm., being only 0.06% of the water equivalent of the calorimeter and contents. It is worth noting here that this close agreement is a further confirmation of the reliability of the writers' Beckmann calibration. It has already been pointed out in the section on differential thermocouples that balanced thermocouples do not necessarily indicate equality of temperature between the jacket and calorimeter when their temperatures are rising at the same rate. A careful investigation in the case of the water calorimeter indicated that this error might

possibly have caused an error of 0.5 gm. in the above water equivalent. If now the calorimeter is to be used for specific heat measurements, the water equivalent, determined electrically, has the very great advantage of including the above errors in such a way as to exclude them from the final result. In this case the calorimeter and Beckmann thermometer become merely transfer instruments.

Thermal Capacity of the Copper Calorimeters and the Specific Heat of Copper (Electrical Experiments)

The thermal capacity of each of the all-copper calorimeters was also determined by the above electrical method, except that both Beckmann and platinum thermometers were used to measure the bath temperature. No weighings were required and no corrections had to be made for air, thermocouples or Beckmann thermometer when the calorimeter was being used in specific heat determinations by the method of mixtures. In fact, the calorimeter, calibrated in this way without corrections, becomes an ideal transfer instrument.

Since these calorimeters are more than 99.5% pure copper, it is evident that we have here a method for determining the specific heat of copper for a small temperature change. This requires, however, the making of all those corrections which are unnecessary when the calorimeter is to be used merely as a transfer instrument. It was because this method of determining the actual specific heat of copper at a definite temperature seemed to be capable of high precision that the platinum thermometer and oil bath were constructed.

The accuracy of this specific heat determination depended upon the measurements of the electrical power supplied, the time, the rise in temperature, and the copper equivalent of the calorimeter which includes the thermocouples, heating coil, and surrounding air. The first three quantities were measured with an accuracy of 0.02% or better. The calculation of the copper equivalent of the No. 2 calorimeter is given below in order to show the size of the various quantities and the probable accuracy. The copper equivalent was obtained by multiplying the mass of each substance by the

	Weight, gm.	Copper equiv., gm.
Copper calorimeter	2953.8	2953.8
Brass hinges and screws	4.1	4.0
Brass tubing for thermocouple	4.6	4.5
Cement for thermocouple	0.12	0.3
Wire for thermocouple	0.4	0.4
Cotton insulation for thermocouple	0.12	0.5
Copper cylinder for heating coil	59.1	59.1
Manganin wire for heating coil	2.5	2.6
Copper lead wires for heating coil	0.3	0.3
Cotton insulation for heating coil	1.1	4.4
Air correction		0.7
Total		3030.6

ratio of its specific heat to that of copper. In spite of some uncertainty in the values of the specific heats of some of the materials, an error of as much as one gram in the total copper equivalent would seem improbable.

In all, over 200 determinations of the specific heat of these copper calorimeters were made at temperatures from $-5°$ to 110° C. The experimental conditions were changed from time to time in order to discover and eliminate any constant errors. The temperature change for a single experiment was usually about four degrees when using a Beckmann thermometer, and eight or ten degrees when using the platinum thermometer. As previously mentioned, the oil bath made possible satisfactory adiabatic conditions at both the low and the high temperatures. The water bath was more convenient between 15° and 40° C.

Specific Heat by the Method of Mixtures ("Dropping Experiments")

Since the application of the adiabatic calorimeter to the determination of specific heat by the method of mixtures is well known, only a few experimental details will be given here.

At the beginning and end of each experiment with the water calorimeter the differential thermocouple was kept balanced for about 30 min. in order to measure the "creep" previously discussed, and a correction was applied in the usual way. When this correction was greater than about 1% the experiment was rejected. In general, no such correction was necessary for the all-copper calorimeters.

In order to make certain that there was thermal equilibrium between specimen and thermocouple, the furnace heating current was always adjusted until the temperature change was less than 0.1° in two minutes. The specimen was then transferred to the calorimeter by holding the furnace directly over the calorimeter and cutting one end of the fine wire supporting it, thus allowing it to fall freely.

Since the thermal capacities of all the calorimeters were determined electrically, the only corrections which had to be considered were those due to the loss of heat of the specimen during the time of fall, about 0.15 sec. Assuming the specimen to be at 400° C., a rough calculation indicated that the maximum loss due to radiation as from a black body would be not more than 0.1%, and that due to conduction not more than 0.05%. The actual loss was undoubtedly much less than this and was partially compensated by the hot air carried into the calorimeter with the specimen. These corrections were so small and uncertain that no attempt was made to apply them. However, an attempt was made to determine the magnitude of this loss experimentally by a difference method. One of the specimens was enclosed in a thin metal sheath and their combined thermal capacities determined and then that of the sheath alone, but the heat losses were too small to be determined in this way.

Experimental Results

Specific Heat of Copper between −5° and 110° C. by the Electrical Method

Table III gives the results (shown graphically in Fig. 7) obtained by the electrical method for the three copper calorimeters, together with some of the experimental conditions. Columns 1 and 2 give the temperature and experimental value of the specific heat in joules per gm. ° C. In most cases these figures represent the mean of two or more determinations made at about the same temperature. Column 3 gives the value of the specific heat calculated from the empirical equations, $7J$, $8J$, $9J$, given in the next section. Column 4 gives the difference between Columns 2 and 3. In Columns 5, 6, and 7 are indicated the bath, thermocouple, and thermometer used in the experiment, *W*, *K*, *O* standing for water, kerosene, and heavy oil baths respectively, 1 and 2 for the earlier and later forms of thermocouple, and *B* and *P* for Beckmann and platinum thermometers.

In the experiments in which thermocouple No. 1 was used, corrections of 0.0001 or 0.0002 have been applied when necessary to correct for errors due to "lag" of thermocouple. Other than this no corrections of any kind were applied to the simple, direct calculations.

TABLE III

SPECIFIC HEAT OF COPPER IN JOULES PER GRAM PER DEGREE C.

Large calorimeter

T°C.	Specific heat		Diff.	Bath	Couple	Thermometer	T°C.	Specific heat		Diff.	Bath	Couple	Thermometer
	Experimental	From equation 7J						Experimental	From equation 7J				
2.8	0.3797	0.3798	−1	W	1	B	44.5	0.3881	0.3882	−1	K	2	P
5.3	0.3805	0.3804	1	W	1	B	46.3	0.3884	0.3885	−1	W	2	P
7.8	0.3814	0.3809	5	W	1	B	47.0	0.3885	0.3886	−1	O	2	P
11.4	0.3821	0.3817	4	W	1	B	48.2	0.3891	0.3888	3	W	1	B
15.5	0.3828	0.3826	2	W	1	B	51.1	0.3895	0.3893	2	W	1	B
18.0	0.3831	0.3831	0	W	2	P	51.4	0.3893	0.3894	−1	O	1	P
18.2	0.3832	0.3832	0	W	1	B	53.9	0.3901	0.3898	3	W	1	B
22.1	0.3841	0.3839	2	W	1	B	54.9	0.3898	0.3900	−2	K	2	P
22.9	0.3841	0.3840	1	W	1	P	56.2	0.3899	0.3902	−3	O	2	P
26.3	0.3848	0.3848	0	W	1	B	57.5	0.3902	0.3904	−2	W	1	B
26.3	0.3848	0.3848	0	W	2	P	62.3	0.3911	0.3911	0	O	1	P
30.5	0.3856	0.3856	0	W	1	B	65.1	0.3913	0.3916	−3	O	2	P
33.3	0.3861	0.3862	−1	W	2	P	67.6	0.3919	0.3920	−1	K	2	P
33.8	0.3860	0.3863	−3	W	1	P	76.0	0.3931	0.3932	−1	O	2	P
34.0	0.3863	0.3863	0	K	2	P	77.9	0.3935	0.3935	0	K	2	P
34.9	0.3865	0.3865	0	W	2	P	87.0	0.3946	0.3948	−2	O	2	P
35.0	0.3870	0.3865	5	W	1	B	91.9	0.3954	0.3955	−1	O	2	P
38.6	0.3875	0.3871	4	W	1	B	96.4	0.3961	0.3961	0	O	2	P
41.9	0.3877	0.3877	0	W	2	P	99.8	0.3965	0.3965	0	O	2	P
44.1	0.3882	0.3881	1	W	1	P	106.6	0.3973	0.3973	0	O	2	P
44.2	0.3882	0.3881	1	W	1	B							

Medium calorimeter

T°C.	Specific heat: Experimental	Specific heat: From equation 8J	Diff.	Bath	Couple	Thermometer	T°C.	Specific heat: Experimental	Specific heat: From equation 8J	Diff.	Bath	Couple	Thermometer
−5.9	0.3779	0.3778	1	K	1	P	38.0	0.3871	0.3872	−1	W	1	B
−0.7	0.3789	0.3791	−2	K	1	P	39.2	0.3875	0.3874	1	W	2	P
1.1	0.3794	0.3795	−1	K	1	P	39.4	0.3875	0.3874	1	W	1	P
3.7	0.3802	0.3801	1	W	1	B	39.6	0.3876	0.3875	1	K	1	P
7.9	0.3812	0.3811	1	K	1	P	39.7	0.3876	0.3875	1	K	2	P
8.6	0.3813	0.3812	1	W	1	B	42.3	0.3880	0.3880	0	W	1	B
13.5	0.3823	0.3824	−1	W	1	B	45.1	0.3885	0.3885	0	W	1	B
15.5	0.3829	0.3828	1	K	1	P	45.1	0.3885	0.3885	0	K	2	P
17.3	0.3832	0.3832	0	W	1	P	45.4	0.3888	0.3885	3	W	1	P
17.9	0.3831	0.3833	−2	W	1	B	46.2	0.3885	0.3886	−1	O	2	P
20.4	0.3839	0.3838	1	W	1	B	46.5	0.3888	0.3887	1	W	2	P
21.3	0.3840	0.3840	0	W	2	P	48.3	0.3890	0.3890	0	K	2	P
21.7	0.3840	0.3841	−1	W	1	P	48.7	0.3891	0.3891	0	W	1	B
24.1	0.3848	0.3846	2	W	1	B	53.3	0.3900	0.3899	1	W	1	B
25.6	0.3849	0.3848	1	K	1	P	55.1	0.3901	0.3902	−1	K	2	P
25.7	0.3848	0.3848	0	W	1	P	58.1	0.3909	0.3907	2	O	2	P
26.4	0.3850	0.3850	0	W	2	P	65.1	0.3918	0.3918	0	K	2	P
28.3	0.3854	0.3854	0	K	2	P	68.4	0.3923	0.3923	0	O	2	P
28.6	0.3854	0.3854	0	W	1	P	74.4	0.3931	0.3932	−1	O	2	P
28.7	0.3855	0.3854	1	W	1	B	78.7	0.3938	0.3938	0	O	2	P
30.0	0.3857	0.3857	0	W	2	P	84.8	0.3946	0.3947	−1	O	2	P
32.1	0.3861	0.3862	−1	W	1	P	89.7	0.3954	0.3953	1	O	2	P
32.4	0.3863	0.3862	1	K	1	P	95.3	0.3960	0.3962	−2	O	2	P
33.3	0.3864	0.3864	0	W	1	B	99.8	0.3967	0.3967	0	O	2	P
33.6	0.3863	0.3864	−1	W	2	P	105.1	0.3973	0.3974	−1	O	2	P
36.5	0.3869	0.3869	0	K	2	P							

Small calorimeter

T°C.	Specific heat: Experimental	Specific heat: From equation 9J	Diff.	Bath	Couple	Thermometer	T°C.	Specific heat: Experimental	Specific heat: From equation 9J	Diff.	Bath	Couple	Thermometer
13.0	0.3817	0.3816	1	W	1	B	40.8	0.3870	0.3870	0	W	2	P
17.3	0.3825	0.3825	0	W	1	B	42.9	0.3875	0.3874	1	W	2	P
19.9	0.3831	0.3831	0	W	2	P	45.1	0.3878	0.3879	−1	K	2	P
21.7	0.3834	0.3834	0	W	1	B	47.9	0.3881	0.3883	−2	W	2	P
23.5	0.3838	0.3838	0	W	2	P	55.4	0.3896	0.3896	0	K	2	P
24.7	0.3841	0.3840	1	W	2	P	65.8	0.3913	0.3913	0	K	2	P
26.0	0.3845	0.3843	2	W	1	B	68.8	0.3915	0.3916	−1	O	2	P
29.4	0.3851	0.3850	1	W	2	P	75.8	0.3932	0.3927	5	O	2	P
30.5	0.3853	0.3852	1	W	1	B	79.0	0.3931	0.3932	−1	O	2	P
32.6	0.3856	0.3853	1	W	2	P	89.0	0.3945	0.3946	−1	O	2	P
34.6	0.3859	0.3859	0	K	2	P	98.1	0.3959	0.3958	1	O	2	P
38.1	0.3867	0.3866	1	W	2	P	106.0	0.3969	0.3968	1	O	2	P

Mean Specific Heat of Tungsten, Molybdenum, and Copper by the Method of Mixtures

Table IV contains a summary of the results obtained for the mean specific heats of tungsten, molybdenum, and copper between widely varying initial temperatures and a final temperature of 28° C. In the actual experiments

TABLE IV

MEAN SPECIFIC HEAT IN CAL_{20} BETWEEN 28°C. AND INITIAL TEMPERATURE

Tungsten

Initial temp., °C.	Calculated from equation 1C	With copper calorimeter	Diff.	With water calorimeter	Diff.	Initial temp., °C.	Calculated from equation 1C	With copper calorimeter	Diff.	With water calorimeter	Diff.
−19.7	.03120	.03124	4			185.6	.03230			.03222	− 8
0.2	.03136	.03128	− 8			200.3	.03235	.03240	5		
54.5	.03171	.03174	3			206.4	.03237			.03231	− 6
64.8	.03176	.03180	4			216.5	.03241			.03250	9
66.2	.03177			.03183	6	238.8	.03248	.03245	− 3		
66.3	.03177	.03167	−10			286.7	.03263			.03257	− 6
68.9	.03179			.03178	− 1	287.8	.03263			.03264	1
71.1	.03180	.03184	4			288.8	.03264	.03264	0		
75.6	.03182			.03171	−11	312.3	.03270	.03268	− 2		
76.3	.03182			.03165	−17	317.5	.03272	.03273	1		
92.1	.03190			.03181	− 9	336.4	.03277			.03267	−10
98.1	.03193	.03197	4			372.4	.03287	.03290	3		
110.4	.03199			.03206	7	417.2	.03299	.03300	1		
133.5	.03209			.03210	1	420.3	.03300	.03304	4		
140.6	.03212			.03201	−11	420.8	.03300	.03304	4		
142.7	.03213			.03203	−10	421.1	.03300	.03298	− 2		
145.9	.03214			.03225	11	422.0	.03301	.03305	4		
151.5	.03216	.03217	1			444.4	.03305	.03304	− 1		
164.9	.03221			.03216	− 5	469.0	.03312	.03309	− 3		
183.4	.03229			.03230	1	500.3	.03319	.03320	1		

Molybdenum

Initial temp., °C.	Calculated from equation 3C	With copper calorimeter	Diff.	With water calorimeter	Diff.	Initial temp., °C.	Calculated from equation 3C	With copper calorimeter	Diff.	With water calorimeter	Diff.
−19.6	.05928	.05925	− 3			226.4	.06310	.06327	17		
00.1	.05974	.05956	−18			234.1	.06317	.06323	6		
50.3	.06073	.06062	−11			235.3	.06319			.06290	−29
64.6	.06097	.06112	15			235.5	.06319	.06353	34		
66.4	.06101	.06111	10			241.1	.06324	.06326	2		
68.6	.06103			.06106	3	274.3	.06358			.06325	−33
75.8	.06115			.06133	18	275.7	.06360			.06329	−31
76.2	.06115	.06114	− 1			309.4	.06393	.06405	12		
83.5	.06128	.06113	−15			316.7	.06400			.06370	−30
91.0	.06138			.06132	− 6	320.3	.06403	.06405	2		
99.9	.06152			.06167	15	320.4	.06403	.06399	− 4		
104.0	.06158	.06156	− 2			372.9	.06452	.06450	− 2		
108.4	.06164			.06162	− 2	414.6	.06488	.06475	−13		
132.6	.06197			.06197	0	418.0	.06491	.06490	− 1		
143.0	.06210			.06167	−43	419.0	.06492	.06488	− 4		
146.3	.06215			.06196	−19	420.5	.06493	.06491	− 2		
153.9	.06225	.06235	10			424.3	.06496	.06501	5		
163.8	.06237			.06238	1	447.8	.06516	.06515	− 1		
190.0	.06268			.06261	− 7	470.3	.06534	.06530	− 4		
219.1	.06301			.06313	12	501.4	.06559	.06569	10		

Copper

Initial temp., °C.	Calculated from equation 5C	With copper calorimeter	Diff.	With water calorimeter	Diff.	Initial temp., °C.	Calculated from equation 5C	With copper calorimeter	Diff.	With water calorimeter	Diff.
−18.5	.09070	.09050	−20			200.0	.09491			.0950	1
0.1	.09122	.09114	− 8			206.0	.09499			.0951	1
0.3	.09122	.09145	23			216.7	.09512			.0954	3
48.5	.09238	.09214	−24			234.9	.09535			.0954	1
52.2	.09245	.09243	− 2			238.2	.09541	.09531	−10		
64.9	.09272	.09264	− 8			238.6	.09541	.09528	−13		
74.3	.09290			.0930	1	273.2	.09582			.0957	− 1
74.8	.09291			.0930	1	275.0	.09584			.0958	0
75.3	.09293	.09303	10			286.5	.09598	.09599	1		
99.1	.09336	.09338	2			290.4	.09602			.0961	1
100.1	.09337			.0937	3	290.7	.09602			.0959	− 1
108.3	.09351			.0936	1	310.8	.09626	.09613	−13		
130.4	.09388			.0940	1	314.8	.09630			.0963	0
144.0	.09409			.0942	1	323.1	.09639	.09622	−17		
151.0	.09420	.09416	− 4			372.1	.09693	.09696	3		
151.2	.09420			.0943	1	414.0	.09735	.09722	−13		
162.7	.09437			.0943	− 1	417.8	.09739	.09742	3		
174.5	.09454			.0951	6	417.9	.09739	.09728	−11		
179.3	.09461			.0950	4	446.4	.09766	.09762	− 4		
185.8	.09470			.0949	2	469.9	.09789	.09796	7		
198.7	.09489	.09499	10			501.0	.09818	.09835	17		

the final temperature often differed from this by several degrees, but the temperature range was easily adjusted without appreciable error, since the specific heat is nearly a linear function of the temperature. The results obtained with the copper and water calorimeters are shown separately in Columns 3 and 5. In Columns 4 and 6 are given the differences between these and the values given in Column 2, which were calculated from the empirical equations, 1*C*, 3*C*, and 5*C*, given in the next section. No differences were detected in the values of the specific heat of the several copper cylinders used.

Equations for Specific Heat

Theory

The Debye (4) function for the specific heat at constant volume can be expanded in a series, the terms of which are multiples of even powers of θ/T, where T is the absolute temperature and θ the "characteristic temperature" of the metal. This expansion is valid for a large range of temperature, including that under investigation. The first two terms in this expansion are $A - C/T^2$. Thermodynamic theory gives for the difference between the specific heat at constant pressure and the specific heat at constant volume an expression approximately equal to a constant times T. It was therefore decided to see how closely equations of the form

$$C_p = A + BT - C/T^2$$

could be made to fit the experimental results.

This equation is directly applicable to the specific heat values given in Table III but not to those given in Table IV, since the latter are the mean values between 28° C. and various initial temperatures. The equation can, however, be readily transformed to apply to mean values as follows:

Let S_m be the mean specific heat between 301° K (28° C.) and $T°$ K, and C_p be the true specific heat at $T°$ K. Then

$$S_m(T - 301) = \int_{301}^{T} C_p dt = \int_{301}^{T} (A + BT - C/T^2)dt$$
$$= A(T - 301) + \tfrac{1}{2} B(T^2 - 301^2) - C\frac{(T - 301)}{301T}$$

and so

$$S_m = A + \tfrac{1}{2} B(T + 301) - C/301T$$
$$= A + 155\tfrac{1}{2} B + \tfrac{1}{2} BT - C/301T$$
$$= A_o + B_o T - C_o/T.$$

Equations from Data by Method of Mixtures

Large-scale *mean* specific heat curves were constructed from the data given in Table IV and the constants A_0, B_0, and C_0 determined by the "cut and try" method. The resulting S_m equations are given below. As indicated in Table IV the average deviation of an individual determination from the curve was about 0.1% when the copper calorimeter was used and about 0.2% in the case of the water calorimeter.

The constants A, B, and C of the C_p equations were then calculated using the above relations between the constants. The resulting equations are given below in 20-degree calories (C – equations) and in joules (J – equations) in order to facilitate comparison with the results of other investigators. Equations 2C, 4C, and 6C are also shown graphically in Fig. 6.

Tungsten

$$S_m = 0.03248 + 0.00000164T - .43/T \quad (1C)$$
$$C_p = 0.03199 + 0.00000328T - 129/T^2 \quad (2C)$$
$$C_p = 0.1337 + 0.0000137T - 540/T^2 \quad (2J)$$

Molybdenum

$$S_m = 0.06250 + 0.0000060T - 1.20/T \quad (3C)$$
$$C_p = 0.06069 + 0.0000120T - 361/T^2 \quad (4C)$$
$$C_p = 0.2537 + 0.0000502T - 1510/T^2 \quad (4J)$$

Copper

$$S_m = 0.09486 + 0.0000068T - 1.50/T \quad (5C)$$
$$C_p = 0.09280 + 0.0000136T - 452/T^2 \quad (6C)$$
$$C_p = 0.3879 + 0.0000569T - 1890/T^2 \quad (6J)$$

Equations for Copper from Data by Electrical Method

The curves 1, 2, and 3, Fig. 7, are plotted from the experimental values given in Table III for the large, medium, and small calorimeters respectively. The lower part of the curve for copper in Fig. 6 is added for the sake of comparison. The following three equations were obtained from large-scale curves by the "cut and try" method.

Calorimeter 1 $C_p = 0.3889 + 0.0000569T - 1890/T^2$ (7J)

Calorimeter 2 $C_p = 0.3891 + 0.0000569T - 1890/T^2$ (8J)

Calorimeter 3 $C_p = 0.3884 + 0.0000569T - 1890/T^2$ (9J)

Calorimeter 3 $C_p = 0.09292 + 0.0000136T - 452/T^2$ (9C)

The differences between the constant terms of equations 6*J*, 7*J*, 8*J*, 9*J* will be discussed in the next section.

Approximate Linear Equations

For some purposes a linear equation over a limited range of temperature is sufficiently accurate and much easier to evaluate than those above. The following linear equations are therefore given merely for practical convenience. They do not differ from the more exact equations 2*C*, 4*C*, and 9*C* by more than 0.2% over the indicated temperature range. C_p is in cal./gm.° C. and t is Centigrade temperature.

Temperature Range 0° to 100° C.

Tungsten	$C_p = 0.03121 + 0.0000113t$	(10*C*)
Molybdenum	$C_p = 0.05926 + 0.0000344t$	(11*C*)
Copper	$C_p = 0.09076 + 0.0000415t$	(12*C*)

Temperature Range 150° to 500° C.

Tungsten	$C_p = 0.03200 + 0.0000047t$	(13*C*)
Molybdenum	$C_p = 0.06150 + 0.0000160t$	(14*C*)
Copper	$C_p = 0.09357 + 0.0000186t$	(15*C*)

Results of Previous Investigators

It seems desirable for purposes of comparison to record here several of the more recently published equations for the specific heats of tungsten and molybdenum, and for the sake of completeness to add the two equations for copper given in the Introduction.

The following three equations for the specific heat of tungsten were published about the time, or soon after, this investigation was started:

$C_p = 0.0319 + 0.000006t$ by Zwikker (13)

$C_p = 0.032036 + 0.00000452921t$ (Range 100° to 900° C.) by Magnus and Holzmann (10).

and $C_p = 0.03199 + 4.848\ 10^{-6}t - 11.74\ 10^{-11}t^2$ (Range 0° to 1600° C.) by Jaeger and Rosenbohm (8).

In 1927 Van Voorhis (12) published a paper on the work function of molybdenum in which he gives a summary of the values of its specific heat, and points out that the divergencies are so great as to render them useless. However, he decides upon the following linear equation as the most probable.

$$C_p = 0.0606 + 0.000028t.$$

More recently two other equations have been published,

$C_p = 0.05973 + 0.00001619t$ (Range 0° to 445° C.) by Stern (11)

and $C_p = 0.0593 + 0.000013\,(t + 40) - 0.0265/(t + 40)^{1.00}$ by Cooper and Langstroth (3).

The range given for the latter is −40° to 250° C. which is obviously impossible.

The following two equations for copper have already been given in the Introduction:

$C_p = .0905 + .000048t$ (Range 0° to 50° C.) by Harper (5),

and $C_p = .0916 + .000025t$ (Range 0° to 500° C.) from I.C.T. "best" values.

All the above equations show the wide divergencies, especially in the second term, which have already been discussed in the case of copper.

Discussion of Results

The three specific heat curves of Fig. 6 have the same general characteristics which have been briefly discussed in the Introduction. That these curves are far from linear and that the change in the slope with the temperature is much greater at low, than at high, temperatures is shown by the figures in Table V.

It is clear from these figures that neither a first nor a second degree equation will fit the experimental curves. It is not possible, therefore, to compare satisfactorily our results with those of others given in the previous section. It is perhaps enough to point out that the high but rapidly decreasing values of the slopes of the curves from 0° to 100° C. account in part for the variations in the second term of the equations obtained by different observers. It is also worth noting that these variations are not in general greater than those between our linear equations (10*C*, 11*C*, 12*C*) for the range 0° to 100° C. and the equations (13*C*, 14*C*, 15*C*) for the range 150° to 500° C.

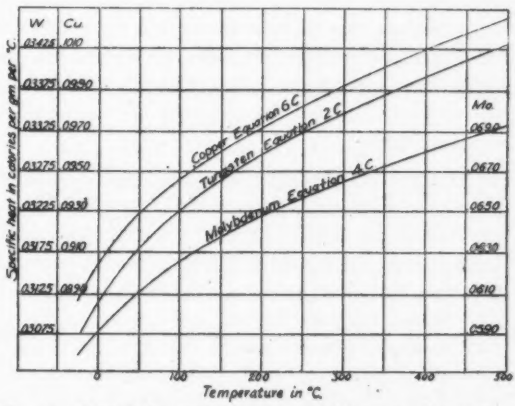

FIG. 6. *Specific heats of tungsten, molybdenum and copper.*

TABLE V

SLOPE OF CURVES

Temperature	0°	250°	500°
Tungsten	0.0000160	0.0000051	0.0000040
Molybdenum	0.000048	0.000017	0.000014
Copper	0.000058	0.000020	0.000016

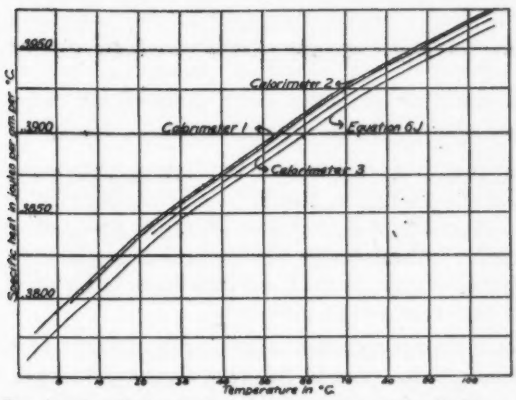

FIG. 7. *Specific heats of copper calorimeters and cylinders.*

It has been pointed out previously that the accuracy of the "dropping experiments" decreases rapidly as the initial temperature approaches the final temperature because of the smallness of the quantities to be measured. This means that the precision is small between 0° and 50° C.

where high precision is especially needed. On the other hand it is for this range of temperature that the "electrical method" is particularly suited, but unfortunately it could be applied only to copper. It is therefore of particular interest to compare the results for copper below 100° C. obtained by the two methods.

These results are shown graphically in Fig. 7 and by equations $6J$, $7J$, $8J$, and $9J$. The four curves were found to be parallel and therefore the B and C terms are the same for the four equations. The fact that no difference could be observed in the shape of the curves obtained by the two methods is a fine check on the reliability and accuracy of the results obtained not only for copper but also for tungsten and molybdenum where only one method was available.

It is next necessary to examine and, if possible, find an explanation for the variation in the constant terms of the four copper equations. It is seen from equations $6J$, $7J$, $8J$, and $9J$ that the specific heats of the two cast-copper calorimeters are nearly the same and distinctly higher than that of the smallest calorimeter which was of cold-rolled copper of about 1% greater density. The consistency of the determinations under widely varying conditions, the fact that the specific heat curves of the three calorimeters are parallel and that all three were used in the determinations of the specific heat of the cylinders, all suggest that the differences in the specific heats are real and not introduced by experimental errors.

No attempt was made to obtain a chemical analysis of the copper, as it seemed very improbable that impurities were responsible for the differences in specific heat. Commercial copper wire and rod are usually over 99.9% pure and, according to the I.C.T., 1% of zinc changes the specific heat of copper only by about 0.01%. It was, however, suspected that the low density and high specific heat of the cast copper might be due to cavities in the castings containing more or less copper oxide. Support was given to this possibility by the finding of one rather large cavity during the machine work on the largest calorimeter. In order to settle this point, a tube containing about 100 millicuries of radium emanation was placed inside each calorimeter and the outside surface covered with photographic film. An exposure of from 15 to 25 min. produced a picture showing clearly every detail of the calorimeter, but there were no signs of any cavities or irregularities in density.

Theory shows that the specific heat is a function of the density and that it would be possible to calculate the change in specific heat corresponding to a 1% change in density if the other physical properties of copper were sufficiently well known. The required constants are too uncertain to give any real significance to such a calculation, but the available data point to a greater difference in the specific heat than that actually observed.

The specific heat of the small copper cylinders is seen to be about 0.15% less than that of the smallest calorimeter, but in this case there is practically no difference in density and both are made from cold-rolled copper rods. It

is natural in this case to suspect that the difference is due to the different experimental methods used, since the electrical method was used for the copper calorimeter and the method of mixtures in the case of the small cylinders. As already mentioned, no correction has been made for the small and uncertain loss of heat from the cylinder during its time of fall. Rough calculations indicate that this loss should be well below 0.1% for temperatures under 100° C. It does not seem, therefore, that this loss can account for the entire difference of 0.15%. It is, of course, possible that some other error of the order of 0.1% has crept into one or the other method of measurement; or variations in some of the physical properties of the two samples of copper may be responsible for the difference.

Equation 9*C* (the specific heat equation for the small copper calorimeter) is selected for the Abstract and as a basis for the approximate linear equations, because the copper of which it is made is more nearly a standard commercial product than copper castings and because it is believed that the "electrical experiments" are more accurate than the "dropping experiments."

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